

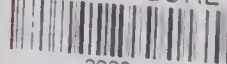
SOUTH PACIFIC COMMISSION
The Chemical Composition of South Pacific Foods

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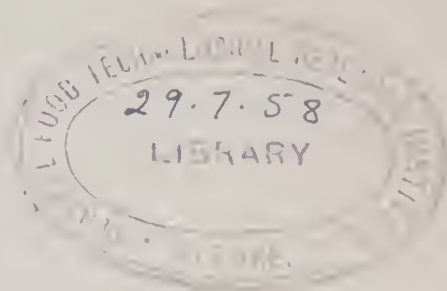


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South Pacific Commission
Technical Paper No.115

THE CHEMICAL COMPOSITION
OF SOUTH PACIFIC FOODS

F.E. PETERS



South Pacific Commission,
Noumea, New Caledonia
February 1958

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PREFACE

To some people the setting up and running of a laboratory for food analysis in a South Pacific island seemed a serious enterprise. It was considered that the high cost involved and continued efforts required would be quite out of proportion with the results achieved. This was even more so as the aim was to conduct an original study of the amino-acid and vitamin contents of local foods which had hardly ever been examined from that point of view.

Certainly there has been no lack of difficulties, but these were all solved thanks to the competence and tenacity shown by Mr. F.E. Peters and his lady assistants, together with the generous and friendly help given by numerous scientific personalities who were convinced from the start that the results hoped for would be of great interest and practical value.

It is impossible to name here all those people who assisted us at the beginning and encouraged us to carry on. However, we are particularly indebted to Messrs. S. Moore and W.H. Stein, from the Rockefeller Foundation, New York; Mr. F. Bugnicourt, Director of the Institut Français d'Océanie and Mr. Tercinier, Head of the Laboratory of Pedology at this organization; and the Secretary and members of the Williams Waterman Fund for the Combat of Dietary Diseases, Research Corporation, New York. The technical advice we received from them, their friendly hospitality and financial help were the main factors which encouraged us to carry on with our work. We beg them to accept the deep gratitude of the South Pacific Commission.

At the end of 1956, just at the time when the laboratory had overcome its teething troubles and work was in full swing, we regretfully were forced to stop. Therefore the results presented by Mr. Peters are per force incomplete. However, we do hope that they will have some usefulness and that, somewhere in the world, other research workers will be encouraged to pursue this task.

Dr. E. Massal
Executive Officer for Health

Original text: French

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I. INTRODUCTION

The contact of the South Pacific islanders with Europeans over the last century or century and a half has resulted in important changes in the life of the peoples and in the economy of the islands they inhabit. Some of them, as in parts of New Guinea, still live on a strict subsistence economy as they have done for centuries, while others who have been more influenced by outsiders have come to neglect the cultivation of their traditional food plants and given most of their efforts and time to the development of cash crops and to occupations for wages. An economic pattern is developing whereby the subsistence of the Pacific islanders is based on the production of some staple foods, supplemented by imported foods purchased from cash income.

In some areas a number of valuable food plants, such as various types of beans, have been introduced by Europeans and Asians, and have been adopted by the indigenous peoples. Such satisfactory additions to the diets have been counterbalanced, however, by the introduction of European cooking utensils and methods which are often inferior, from the nutritional standpoint, to the traditional methods in preserving the bulk of the nutrients.

A change-over from a subsistence to a cash economy and from traditional practices to European methods of food preparation cannot be avoided. It should be the responsibility of territorial administrations to assist the peoples in the transition, to improve existing nutritional standards where these are not as high as they could be, and to attempt to maintain 'adequate diets and nutrition'. The points requiring particular attention are :

- (a) The research among traditional food plants for species and varieties susceptible to improvement and which can be retained in the new agricultural patterns;
- (b) the development of production of "rich" foods;
- (c) the education of the islanders in the relative values of the different foods, local or imported, and in sound methods of preparation.

The dietary and nutritional problems of the South Pacific region are complex, and differ within the area. The coral atolls, the high volcanic islands, the large land masses of New Guinea, each have their peculiar set of problems. The rapid development of relatively large urban populations also raises special questions.

Since the end of the second World War efforts have been made by different organizations to investigate some of the problems. In particular, the Australian Government organized a full-scale nutrition survey in Papua and New Guinea in 1947, and two organizations - the South Pacific Health Service and, on a larger scale, the South Pacific Commission - have developed long-term research programmes. Further, localized dietary and nutritional

surveys have been carried out by governments or scientific institutions.

The need for chemical analyses of foodstuffs was felt in the early stages. During the Australian survey in New Guinea selected foods were collected and analysed at the Australian Institute of Anatomy, Canberra. Moisture, protein, fat, available carbohydrate, crude fibre, ash, calcium and ascorbic acid were evaluated.

The investigations carried out by the South Pacific Commission into the diets and nutritional status of the indigenous peoples and into their subsistence economy called for a wider knowledge of the composition of staple and auxiliary foods. The larger uses of local foods and the breeding of nutritionally-improved varieties could not be recommended unless supported by the results of chemical analyses.

After a transitory period during which food analyses were performed at the Australian Institute of Anatomy, the Commission decided in 1952 to establish its own food analytical laboratory which began to function in 1954. Operations were suspended at the end of 1956.

II. ESTABLISHMENT OF LABORATORY

When the Commission undertook to engage in food analyses it was first hoped that such activities would be carried out in an already established laboratory, and approaches were made to several institutions to undertake this work on Commission's behalf or to provide space and facilities for a Commission worker in this field.

The space and facilities offered in the early days by the Australian Institute of Anatomy, Canberra, were no longer available in 1952. But towards the end of 1952, the Institut Français d'Océanie at Noumea, the Pacific branch of the Office de la Recherche Scientifique et Technique Outre-Mer, indicated that it would be prepared to offer satisfactory space in the soil chemical laboratory that was being built. Work began early in 1953 and the laboratory was ready for occupation in January 1954.

The Commission's approved programme concentrated on the analysis of staple foods for proximate composition and for amino-acid content of the proteins. The work was later expanded to include analyses of other foods and vitamin assays.

Whilst the laboratory was being built, the Commission's biochemist was sent on a study tour in the United States of America and elsewhere to familiarize himself with the latest techniques for amino-acid and vitamin analyses. Visits to European and South Asian laboratories were also included in the tour, so that the biochemist could establish the necessary scientific contacts and could be aware of the programmes and achievements of other workers in this field.

On his return to Noumea, the biochemist proceeded to organize the laboratory and assemble the equipment, some of which, as well as certain analytical chemicals, had already been obtained. Further apparatus and supplies were now procured.

Many delays in starting work were occasioned by the non-arrival of critical reagents or equipment, and the analyses began piecemeal, specific estimations being carried out as the supplies became available.

In May 1954 a New Caledonian assistant was recruited and trained to perform simple analyses. Unfortunately, when she was becoming useful, she was obliged to resign her post. Another assistant was appointed in March 1955. She was of above normal intelligence and rapidly picked up laboratory techniques and practices. She proved an invaluable assistant. In April 1956, Miss P. Wills, a Sydney graduate, was appointed to assist with the vitamin analyses.

Difficulties were experienced with the installation of equipment. Especially was this true with the delays in delivery of the fraction collector for amino-acid estimates, and with the spectrophotometer. This latter instru-

ment arrived from England in a damaged condition. Although many of the faults were rectified in Noumea, it was necessary to ship the instrument to Sydney for repair. Finally a French electronic engineer visiting Noumea was able to put the instrument in full working order. Altogether a whole year elapsed between the delivery of this piece of apparatus and the time when it was finally ready for use. Time delay in this case was an extreme example, but similar delays and frustrations occurred with other equipment.

Work was nevertheless carried out, but it was only in May 1956 that the laboratory was fully staffed and adequately equipped to function efficiently. Then, the Commission decided to discontinue food analysis work in December 1956.

III. METHODS EMPLOYED

The methods used for the collection and preservation of the food samples and for the various analyses are described below. In many cases modifications were made in existing methods and in these cases the method used is described in some detail.

1. COLLECTION AND PREPARATION OF SAMPLES

The samples received for analysis came from many of the territories of the South Pacific although the majority were obtained in New Caledonia. The procedure used for preservation during shipment was mainly that devised by Munsell et al. (1)

The following instruction was distributed to all who might collect samples:

"COLLECTION AND STABILIZATION OF FOOD SAMPLES"

1. When collecting samples of food of plant origin it is important to obtain information on the conditions under which they were grown and harvested. The best samples, therefore, are those gathered directly from the field. Further, since plants of the same species but of different strain or variety may differ considerably in nutrient content, it is important that the food being sampled be accurately identified botanically. Only specimens of a single strain or variety should be used in preparing a sample for analysis. Mixing specimens of different varieties in one sample should be strictly avoided.

2. When gathering or buying food for sampling, it is important to obtain enough so that a sufficient number of items may be included, in whole or in part, in the final sample to make it representative. One or two items are not enough for a sample however much they may weigh. An attempt should also be made to select items of uniform quality for any single sample. As soon as the items for a sample have been selected, a description (size, degree of maturity, quality, etc.) should be derived and recorded.

Representative sampling of each plant material is, to a degree, an individual problem. It is not feasible to outline a general plan that will apply to all foods. The one objective is to obtain a portion, the subsample, that is representative of the whole. There are various ways of doing this dependent upon the nature of the material being handled. A few illustrations are given:

- a) Roots: slices may be cut perpendicular to the long or the short axis; these slices are then bisected or even cut in small pieces. The entire amount so obtained is thoroughly mixed and the subsample taken from the mixture.
- b) Leafy vegetables: successive leaves are peeled from the head and placed successively in fifteen piles (reversing from left to right to left, etc.) until all of the leaves from the head are distributed. Any pile may then be taken as a representative sample.
- c) Fruits and other fleshy foods: wedge-shaped slices may be taken from several items and mixed. In cutting the slices care should be taken that they are representative of the whole. For most fruits the slices should be taken from stem end to blossom end.
- d) Seeds, legumes, etc.: foods consisting of small units such as seeds present little difficulty for subsampling provided the original sample consists of specimens of a single strain.

For cutting and mixing foods, stainless steel knives and spoons should be used and mixing should be done in glass or enamelware dishes. If enamelware is used it must be free of blemishes showing exposure of the metal surface.

No food should be sampled until the specimens to be used have been thoroughly cleaned and the inedible parts removed. The part considered edible may depend somewhat on practice and personal preference. An exact description of the method of preparing the food for subsampling must be given so that it may be used in connection with the appraisal of the analytical results.

In order to make a complete analysis of a sample it is generally necessary to hold it for some time. Staple foods such as cereals, legumes, nuts, etc., when they are sufficiently dry, may be held in tightly closed metal containers. Samples of perishable foods must be stabilized.

A satisfactory medium for this purpose has been found to be a solution of oxalic acid in water for subsamples to be used in the determination of all constituents except carotene. The stabilizing solution consists of an accurate 1% oxalic acid solution. The subsample is prepared by blending 200 g. of food with 100 ml. (100.35 g.) of the 1% oxalic acid solution.

The stabilized samples (subsamples) are best prepared by using a Waring Blendor (milk shake mixer), but well-chopped samples will be satisfactory. Bottles of brown glass are used for storing the subsamples. Since any inaccuracies in weights used in making the subsamples are reflected in the analytical results, the amount of food and of stabilizing solution used for the slurries must be measured precisely and the slurry must be of as uniform consistency as it is possible to obtain compatible with the nature of the food. In the determination of moisture a correction will be made to take account of the weight of the oxalic acid in the dry material and the 1% oxalic acid stabilizing solution must be accurately prepared.

"PREPARATION OF THE SUBSAMPLE"

If a Blendor is used, the 200 g. of food is weighed out and must be in sufficiently small pieces so that they will not stall or clog the Blendor. For most fruits and vegetables the food is weighed in a large beaker and transferred in toto to the Blendor cup. The oxalic acid solution is then weighed out in the same beaker and added to the food. In some cases it may not be advisable to start the blending with the entire amount of food in the cup but to place the acid solution in the cup first with a portion of the sample and then add the remainder of the food as the mixture becomes blended. Blending should continue until the mixture is homogeneous but not long enough for it to become more than warm (2-3 min.). When blending is complete any solution or slurry on the cover and sides of the cup must be added to the main contents of the cup so that the whole represents a uniform mixture. If it is necessary to scrape down the sides of the cup, a rubber policeman or stainless steel spatula may be used but any portion so removed must be mixed with the main mass. The slurry is then transferred to the brown glass bottle by a quick pouring motion and the cover to the bottle is screwed down tightly.

In some cases the texture of the food may be such that a higher proportion of the oxalic acid solution to food is required to give a satisfactory blend. When using additional oxalic acid solution it is advisable to use only such an amount as will give a satisfactory blend. Care should also be taken that the weights of the food and of the oxalic acid solution used give a reasonable dilution factor.

It is realized that in most places Blendors may not be obtainable. In this case the samples must be cut up as fine as possible and immersed in the oxalic acid solution. They will then be blended immediately on arrival at the laboratory.

The subsample slurries are stored in a refrigerator until used. If the bottled samples are to be shipped any distance, the bottles should be sealed with a pliant sealing wax. If the subsample is held for any length of time, care must be taken that portions are not left on the cover or sides of the bottles where they may dry out. If such portions have been left and have dried out they should not be brought into the main slurry.

2. PROXIMATE COMPOSITION

The methods and techniques used in establishing the proximate composition of the food samples are described below in detail.

A. WATER. Water was determined in all the food samples by the entrainment distillation method using toluene as the distilling agent. Winton (2).

About 10g. of samples were placed in a 250 ml. round bottomed quick-fit flask, which was connected to a 7.5 ml. graduated Dean and Stark tube fitted with a condenser. Toluene was added down the condenser to fill the tube and cover the sample and the whole was heated until the water

stopped distilling over. The apparatus was allowed to come to air temperature and the amount of water present read off directly from the graduated tube. The samples were run in duplicate.

B. CRUDE FIBRE. The crude fibre method used was that suggested by van de Kamer and van Ginkel (3).

In this method 1 g. of the sample was digested for exactly 30 minutes with ten times its volume of Scharrer-Kürschner reagent in a 150 ml. acetylation flask having a quick-fit joint and fitted to a 100 cm. air condenser. It was then cooled under the tap. 1.5 times the volume of diisopropyl ether was added and the contents shaken and allowed to separate. The ether layer was decanted through a sintered glass Buchner funnel. The process was repeated twice. 25 ml. of the cold reagent were then added and the whole contents transferred to the Buchner funnel and washed three times with 10 ml. of cold reagent. The contents of the Buchner funnel were then washed with hot water until acid free to litmus. A 100 ml. Erlenmeyer flask containing 25 ml. of the dichromate-sulphuric acid solution was placed in a large jar fitted with a large (No.15) rubber stopper having two holes bored through it. A glass tube passed through one hole and led to the water pump, into the other hole the Buchner funnel was inserted so that the stem projected into the Erlenmeyer flask. 5 ml. of concentrated sulphuric acid were placed on the filter and stirred until the crude fibre had dissolved; the acid was then drawn through into the Erlenmeyer flask by suction. This was repeated 5 times.

The Erlenmeyer flask was then heated in a boiling water bath for exactly 10 minutes. After cooling the contents were transferred to a 500 ml. volumetric flask and made to 500 ml. with water. N.Potassium iodide was added to an aliquot of this solution and the excess dichromate was titrated against 0.1 N sodium thiosulphate. Most times it was not necessary to use a starch indicator as the colour change from yellow-brown to green was quite sharp.

The estimation was run in duplicate, together with a blank.

Reagents used:

Scharrer-Kürschner reagent. Dissolve 25 g. trichloro-acetic acid in 600 ml. 70% acetic acid. Add 62 ml. 65% nitric acid (density 1.4) and make to one litre with 70% acetic acid.

Acid Potassium dichromate solution. Two solutions were used depending on the amount of fibre in the sample.

(i) 0.2N. Dissolve 2.45 g. potassium dichromate in 160 ml. water and add 120 ml. 36N. sulphuric acid.

(ii) 1.2N. Dissolve 14.7 g. potassium dichromate in 160 ml. water and add 120 ml. 36N. sulphuric acid.

Sodium thiosulphate solution. 0.1N.

Potassium iodide solution. 1.0N.

Calculation.

$$\% \text{ Crude fibre} = \frac{(t_2 - t_1) \times N \times 6.86 \times V}{P} \times 100$$

where t_1 = ml sodium thiosulphate used by test solution

t_2 = ml sodium thiosulphate used by blank

N = titre of sodium thiosulphate.

V = total volume of test solution divided by the aliquot taken.

P = weight of sample in milligrams.

6.86 = factor for calculating the cellulose content from the dichromate used.

Observations. This method was simpler than the standard acid-alkali method, gave consistent results and the results obtained agreed generally, with those found by other workers.

C. ETHER EXTRACT. The ether extract was determined using the standard Soxhlet method. Di-isopropyl ether was used as the extracting solvent. "Alundum" extraction thimbles were used as these had the advantage of being fire proof and could be used indefinitely.

D. ASH. The ash was determined by ignition of the residue from the ether extraction at 550° C in a muffle furnace. The extraction thimbles from the ether extract were used directly in the furnace.

E. TOTAL NITROGEN. The Conway (4) micro-diffusion method was used. 0.5-1.0g. of sample was digested with about 10 ml. of a digestion mixture, composed of 320 g. potassium sulphate, 500 ml. 36N. sulphuric acid and 1 g. of selenium, until it was clear. After cooling, the reaction mixture was made to 50 ml. with water. 0.5 ml. was pipetted into one side of the outer chamber of a "Conway No.1 unit"; after five minutes 1 ml. of the boric acid solution was placed in the centre chamber. The glass cover, which had a coating of petroleum jelly was partly placed over the unit and 1 ml. of 40% potassium hydroxide was pipetted into the outer chamber opposite the reaction mixture. The cover was placed in position and the contents of the outer chamber were mixed by gentle rotation. The units were left on the bench for 1 hour, after which time the covers were removed and the ammonia trapped in the centre chamber was titrated against N/50 hydrochloric acid using a Conway microburette.

Reagents.

Boric acid solution. 10 g. boric acid A.R. were mixed with 200 ml. ethyl alcohol and 400 ml. distilled water. After the boric acid had dissolved 20 ml. of indicator solution was added and the volume made to 1 litre with distilled water.

Indicator solution. 33 mg. of bromocresol green and 66 mg. of methyl red were dissolved in alcohol, mixed and made to 100 ml.

Calculation.

The total nitrogen content of the material is given by

$$\frac{R \times 0.28 \times V}{v \times w \times 1000} \times 100$$

where R = burette reading in ml. of N/50 HCl.

V = total volume of reaction mixture

v = volume of reaction mixture in unit

w = weight of material taken in grams

0.28 = nitrogen factor.

for most purposes this became $\frac{R \times 0.28 \times 10}{w}$

F. CARBOHYDRATES. Starch and soluble reducing sugars were determined by a modification of the A.O.A.C. methods (5).

The sample was extracted with 60% ethanol in a soxhlet for 12 hours. The alcoholic extract was used for reducing sugars and the residue for starch determinations.

- (i) Reducing sugars. The alcoholic extract was made to 250 ml. with 60% ethanol and an aliquot, usually 50 ml. was evaporated on a water bath. Small quantities of water were added from time to time until the smell of alcohol ceased, a further 20 ml. of water was then added and evaporation continued until about 10 ml. of solution remained. 5 ml. of saturated lead acetate were added to precipitate interfering substances and the whole made up to 50 ml. and filtered. Solid sodium oxalate was then added to the filtrate until precipitation ceased. The precipitate was then filtered off and the filtrate used for the sugar determinations.
- (ii) Starch. The residue remaining from the alcoholic extraction was transferred to a 500 ml. quick-fit round bottomed flask; water and hydrochloric acid of specific gravity 1.125 were added in the proportions of 10 to 1 - the actual amounts added depended on the weight of the residue - and the whole refluxed for 4 hours. The

reaction mixture was cooled, neutralized with solid sodium hydroxide and made to 500 ml. with distilled water. The solution was then filtered and aliquots used for the determination of the reducing sugars formed. The percentage of reducing sugar was multiplied by 0.9 to convert it to percentage starch.

Estimation of reducing sugars. The Somogyi-Schaffer-Hartmann method for blood glucose was adapted for use with the reducing sugar solutions.

5 ml. of copper reagent was pipetted into four 6" x 1" boiling tubes and 5 ml. of sugar solution, 3 ml. of sugar solution plus 2 ml. of water, 2 ml. of sugar solution plus 3 ml. of water and 5 ml. of water respectively were added to each one of the tubes. The solutions were mixed gently, capped with a small filter funnel and placed in vigorously boiling water for 15 minutes. The tubes were then cooled to 35° - 40°C and 1 ml. 5N. sulphuric acid added and the tubes shaken very gently. The tubes were allowed to stand for at least two minutes then titrated with 0.005N. sodium thiosulphate using a starch indicator.

The concentration of reducing sugar was determined by reading from the following table the figure obtained by subtracting the titration figures from the blank.

TABLE 1
Amount of glucose (mg) in 5 ml. of sample.

Millilitres of 0.005 N thiosulphate	Tenths of 1 ml. of 0.005N of sodium thiosulphate									
	0	1	2	3	4	5	6	7	8	9
0	..	..	0.105	0.115	0.130	0.145	0.155	0.170	0.180	0.195
1	0.205	0.220	0.230	0.245	0.255	0.265	0.280	0.290	0.305	0.315
2	0.325	0.340	0.350	0.360	0.375	0.385	0.400	0.410	0.420	0.430
3	0.445	0.460	0.470	0.485	0.495	0.505	0.515	0.530	0.540	0.550
4	0.565	0.575	0.585	0.595	0.605	0.620	0.630	0.640	0.650	0.660
5	0.675	0.685	0.695	0.705	0.715	0.730	0.740	0.750	0.760	0.770
6	0.785	0.795	0.805	0.815	0.825	0.840	0.850	0.860	0.870	0.880
7	0.895	0.905	0.915	0.925	0.935	0.950	0.960	0.970	0.980	0.995
8	1.005	1.015	1.025	1.035	1.045	1.060	1.070	1.080	1.090	1.105
9	1.115	1.125	1.135	1.150	1.160	1.170	1.185	1.195	1.205	1.215
10	1.225	1.240	1.250	1.260	1.270	1.280	1.295	1.305	1.315	1.325
11	1.335	1.350	1.360	1.370	1.380	1.395	1.405	1.415	1.425	1.440
12	1.450	1.460	1.470	1.480	1.495	1.505	1.515	1.525	1.540	1.550
13	1.560	1.570	1.580	1.590	1.605	1.615	1.630	1.640	1.650	1.660
14	1.670	1.685	1.695	1.705	1.715	1.725	1.735	1.750	1.760	1.770
15	1.780	1.795	1.805	1.815	1.825	1.835	1.850	1.860	1.870	1.880
16	1.890	1.905	1.915	1.930	1.940	1.950	1.960	1.970	1.980	1.990
17	2.000									

Reagents used:

Copper reagent. 12 g. sodium potassium tartrate, 20 g. anhydrous sodium carbonate and 25 g. sodium bicarbonate were dissolved in 500 ml. water (1); 6.5 g. copper sulphate $5H_2O$ dissolved in 100 ml. water was added to solution 1 with stirring. 10 g. potassium iodide, 800 g. potassium iodate and 18 g. potassium oxalate were dissolved in 200 ml. water and added to the main solution, which was then diluted to 1 litre with water.

Sodium thiosulphate solution. The 0.005N sodium thiosulphate solution was made as required by diluting a stock solution of 0.1N sodium thiosulphate.

G. CALCIUM. The method used for the determination of calcium was the oxalate precipitation method followed by titration with potassium permanganate (A.O.A.C.)

Attempts were made to determine calcium using the method of Diehl et al. (6) using sodium versenate (sodium ethylene diamine tetra acetate) with an ammonium purpurate indicator. However, the colour changes were not sufficiently distinct to give good reproducible results.

H. PHOSPHORUS. Phosphorus was determined volumetrically by the Kilgore modification of the Pemberton method (4).

3. AMINO ACIDS

The method used for the determination of amino acids was basically that described by Moore and Stein (7 and 8). The method is described below in some detail as modifications were made throughout and the method presented is not necessarily the same as that described in the above papers.

A. Apparatus employed

(i) Fraction collector. During visits to the United States and England several types of fraction collectors used in different laboratories were studied. It was soon apparent that for the successful operation of the column chromatographic technique in Noumea a simple, but efficient machine would be required.

The type recommended by Dr. Moore was a commercial "Technicon" machine which was electrically operated and used either a drop counting device or a simple time relay to measure the volume collected. In either case the apparatus consisted of an electrically operated turntable placed beneath the column of resin. In the drop counting model the drops from the column were passed before a photo electric counter. After the passage of a predetermined number of drops (equal to one or two millilitres) a circuit was activated which caused the turntable to turn a distance such that the next

empty tube was beneath the collector. In the time delay model, after a certain predetermined time period had elapsed (such that one or two millilitres of solution would pass off the column) a circuit was activated and the turntable turned to the next empty tube.

Experience with both these types showed that constant breakdowns were liable to occur which necessitated the presence of a highly qualified electronic mechanic.

After observing many modifications of these two types, it was arranged that an electric collector be built which would work from a simple make and break circuit connected to a syphon. In this the effluent would collect in a glass syphon fitted with two wires welded into the syphon $1/3$ rd and $2/3$ rd up the side. These syphons could be made to hold either 1 or 2 ml. When the syphon was $2/3$ rd full, current would pass through a circuit which would turn the turntable. After delivery, the syphon would remain over the same tube until the syphon was again $2/3$ rd full. This would ensure that the last drops adhering to the syphon outlet would be collected with the remainder of the fraction. The Australian National University contracted to make a machine of this type to hold 90 x 18 mm. tubes.

After a considerable delay the Australian National University advised that they had not the time to experiment with a fraction collector of this type and would supply in its place a "Phillips" flotation type (9). On arrival this machine was found capable of holding only 45 x 12 mm. tubes. Australian National University then agreed to supply a model holding 90 x 18 mm. tubes. This machine consisted of a rack of two discs of $1/16$ " stainless steel drilled just inside their peripheries with 90, 18.5 mm. holes, and held apart by four short pieces of $1/8$ " rod. Through the centre of the discs passed a length of $3/16$ " rod, the top of which was held vertically by passing through a ball-bearing race set into a piece of rectangular section rod. The discs were mounted in a copper tank containing water which rested on a wooden base board. The rectangular section rod extended from a position above the centre of the tank to a retort stand rod mounted on the wooden board. A string was attached to a pulley on top of the $3/16$ " rod which passed over another pulley at the end of the rectangular rod and had some lead weights attached to its free end. The string was wound around the inner pulley until the weights were at their highest position, and when released, the force of gravity on the weights caused the rack to rotate.

The colorimeter tubes were inserted in the holes in the discs in such a way that they should bob up and down freely in the water with no sideways movement if touched lightly. The rack was prevented from rotating by a strip of safety razor blade projecting in front of one of the tubes. A chromatographic column was mounted above the tube held by the razor blade. As the solution came off the column it collected in the tube which slowly sank in the water until it passed under the razor blade. The weight on the string then caused the rack to rotate until the next tube was stopped by the piece of razor blade.

This was the theory of operation. However, when the machine was assembled in Noumea, it was found that because of the weight of the steel discs a considerable weight was required to rotate the rack. This heavy weight resulted in there being a high degree of friction between the tube and the razor blade stop. It was also found that the weight of the colorimeter tubes was such that the addition of 2 ml. of sample from the column, coupled with the friction, was not sufficient to cause the tubes to sink, and at least 5 ml. and often 10 ml. had to be added. Various procedures were adopted to try and overcome these problems, including the addition of detergents to the water, replacement of the water by ethyl alcohol, petrol, petroleum ether, light oils, etc., but without very marked success. The razor blade was replaced by other materials. The best results were obtained using acetone as the supporting fluid and a fine glass filament as the stop. However, this was not entirely satisfactory and the glass filament tended to be extremely fragile.

Eventually a machine similar to the model 2 of James, Martin and Randell (10) was built in Noumea. This consisted essentially of a syphon mounted on a counter-balanced arm which was pivoted about its centre. In filling and emptying this syphon could be made to operate a mechanical escape mechanism.

The machine, as built, consisted of a sheet of $1/8$ " masonite, 36" x 40", which had a hole 31" in diameter cut from the centre, 90 holes $18\frac{1}{2}$ mm. in diameter were drilled at 4° intervals around the outside of the hole to accommodate the colorimeter tubes. Between each colorimeter tube a pin consisting of a piece of stainless steel wire 2" in length projected inwards toward the centre. The piece of masonite was held rigid by pieces of 2" x 2" board running along the undersurface of the edges, and the whole was held at the four corners over a piece of reinforced plywood at a height such that about $1\frac{1}{2}$ " of a 6" tube projected through the $18\frac{1}{2}$ mm. holes.

Projecting through the centre of the plywood base was a 10" piece of hard steel rod ground to a point at the top, which acted as a pivot. The counter-balanced arm was made of brass and consisted of a central piece of $3/8$ " square rod which had a punch mark to fit the pivot. Brazed into one end of this rod was a piece of $3/16$ " round rod $12\frac{1}{2}$ " long to support the syphon, and into the other end a piece of $3/16$ " round rod $13-3/4$ " long to hold brass counter-balances. A piece of $1/4$ " round rod $3\frac{1}{2}$ " long was brazed into the underside of the square rod adjacent to the counter-balancing arm. This piece of $1/4$ " rod held a sliding weight for the adjustment of the centre of gravity vertically. To the bottom of the $1/4$ " rod was brazed a piece of plate $2-3/4$ " x 1" from which an ellipse $1\frac{1}{2}$ " x $3/8$ " had been drilled. Two pieces of $1/4$ " square rod were brazed to this plate, and through the square rod passed excursion-limiting screws.

A piece of $3/16$ " round rod was brazed vertically below the extreme end of the rod supporting the syphon. Another piece of $3/16$ " rod was brazed on to the bottom of this latter rod and on to the brass plate, and it held at its extreme end two pieces of stainless

steel wire which formed the escapement mechanism with the radial pins.

The whole of the counter-balanced assembly was caused to rotate by using a spring made of soft chrome-iron wire which had one end fixed into the central piece and the other end fixed into a brass collar which could be tightened on to the steel pivot rod by a thumb screw.

The escapement was operated by bending the stainless steel wires to form a square or loop, but with the ends overlapping slightly. When the syphon was empty the lower or upward pointing piece of wire was pressed against one of the radial pins. As the syphon filled the beam slowly tilted and the loop descended and the pin passed between the two ends. The arm was now free and rotated because of the torque generated by the spring, until the upper or downward pointing piece of wire came in contact with the next radial pin. When the syphon emptied the beam was tilted back and the upward pointing end of the loop rested against the pin, as in the starting position. The syphon would move forward a distance equal to that separating the ends of the loop. This distance was however quite small and the remaining drops left in the syphon delivery arm would empty into the same tube.

The collecting cup and syphon were made of glass and were connected by lengths of capillary tubing joined by tygon tubing. The cup was mounted on the central piece directly over the pivot and was held in place by two clips fitted at the ends of the brass syphon support, which clipped on to the capillary tubing joining the syphon and collecting cup. The syphon was made adjustable by passing a piece of capillary tubing into it through a tygon tubing collar. Raising or lowering the capillary tubing increased or decreased the capacity of the syphon.

Air bubbles were prevented from entering the capillary tubing from the collecting cup by placing a small piece of cotton wool on the bottom of the cup.

The positions of the counter-balances were found to be critical. The quality of the apparatus was probably inferior to a similar one made in Australia and so frictional forces were relatively high. As only 2 ml. fractions were collected, it was found that the counter-balances had to be such as to give a maximum of upward thrust after the syphon emptied, but would also allow the escapement mechanism to work. A positioning was eventually found that allowed the escapement to operate just before the syphon filled completely or about five minutes before it emptied.

After about six or eight runs the whole apparatus had to be dismantled and thoroughly cleaned, because corrosion tended to occur on the metal parts and this increased the friction at the pivot.

To mount the column, two wooden supports 6" wide and 62" high were screwed toward the back of the collector and were joined across

the top by a piece of wood 6" x 40". To the top piece of wood, retort stands were bolted, projecting both up and down. The column was clamped into position from these retort stands and various reservoirs were also fastened using these retort stands.

A simple U-tube mercury manometer was mounted on to the side of one of these vertical wooden supports. This enabled the pressure on the column to be ascertained, and adjusted if necessary.

- (ii) Chromatography columns. The resin was supported in uniform bore glass tubes 175 cm. long, 0.9 cm. diameter. Into these tubes, five cm. from the bottom was welded a No.0 sintered glass disc. A portion of the bottom of the tubes was drawn out to make a dropping point.
- The tubes were enclosed in demountable condenser jackets 50 cm. long, fitted with bakelite nuts and joined by pieces of tygon tubing. Water at a constant temperature could then be made to pass along the length of the columns and so keep them at the desired temperature.
- (iii) Colorimeter tubes. The same tubes were used to collect the fraction and develop the colour - 18 mm. test tubes without rims. These were purchased as being optically matched, but were standardized and marked using a solution of methyl red in 0.01 N. Hydrochloric acid. After use the tubes were cleaned by filling with a dilute solution of "Teepol" and left standing for 10 minutes. The tubes were then washed until all traces of "Teepol" had been removed, rinsed with distilled water and dried in an oven at 100°C.
- (iv) Buffer reservoirs. The buffers were delivered to the columns from separating funnels which had had the stems pulled down to fit 1/8" tygon tubing. The tygon tubing was connected to a glass U-tube, one end of which passed through a rubber stopper that fitted into the top of the chromatography tube. The U-tube and chromatography tubes were fitted with glass lugs and rubber bands were passed around the lugs to keep the rubber stopper from coming out when pressure was applied.

The stop cocks of the separating funnels were held in place when pressure was applied by using Fisher stop cock clamps.

The mixing apparatus used when the pH and ionic strength of the buffer was increased, consisted of a quick-fit wash bottle which contained a plastic covered Alnico bar and was supported over a magnetic stirrer. The inlet tube of the wash bottle had been bent vertically and was connected to a separating funnel by a piece of 3/8" tygon tubing which contained a length of 3/8" O.D. capillary tubing. The outlet tube of the wash bottle was drawn down and connected to a piece of 1/8" tygon tubing which went to the column.

The wash bottle was filled with pH 3.1 buffer, and the male joint inserted. The wash bottle and connecting tubes were then completely filled with the pH 3.1 buffer, by adding some buffer to the

separating funnel, clamping off the outlet tube of the wash bottle. opening the stop cock of the separating funnel and applying gentle suction at the top of the funnel. Air was withdrawn and the buffer came in to take its place. When all the air had been completely removed the stop cock was closed and excess buffer in the separating funnel was removed with the aid of the water pump. The funnel was then filled with pH 3.1 buffer. The length of capillary tubing between the funnel and the wash bottle prevented the upward flow of the less dense pH 3.1 buffer into the upper reservoir when the stop cock was opened. The magnetic stirrer gave more uniform mixing of the buffers.

- (v) Positive pressure. Positive pressure was maintained by using compressed oxygen. Low pressure gas was delivered with the aid of a reduction valve. The actual pressure delivered to the column was determined using a mercury U-tube manometer.
- (vi) Photoelectric colorimeter. The developed tubes were read at 570 mμ using a "Unicam" absorbtimeter. One modification was made to the instrument. Instead of a $1\frac{1}{2}$ V special dry cell and a $67\frac{1}{2}$ V radio dry cell, a combination $1\frac{1}{2}$ V, 69 V radio dry cell was used. The instrument was quite steady and gave reproducible results providing the 12V accumulator was maintained at a 75% charge level or better.
- (vii) Constant temperature. The columns were run at constant temperatures of 30° C, 50° C and occasionally 75° C. The constant temperature was maintained using a thermostated water bath into which a small water circulating pump had been placed. The condenser jackets surrounding the columns were filled with water by syphoning, and the flow of water was maintained using the small pump.

B. Method

Preparation of the resin. The resin used was Dowex 50 minus 400. Initially 4% crosslinked and 4% + 5% crosslinked resins were used but it was eventually found that 8% crosslinked resin gave the best results. This point will be discussed in more detail later.

One pound of the resin as supplied was washed with 4 to 8 litres of 4N. hydrochloric acid on a Buchner funnel using very gentle suction. The filtrate was initially yellow but became colourless during the washing process. Washing with the hydrochloric acid was discontinued after the filtrate became colourless. The resin was then washed with 5 litres of distilled water followed by 3 litres of 2N. sodium hydroxide. The sodium salt of the resin so formed was then suspended in 3-4 times its volume of N. sodium hydroxide and heated on a waterbath for $2\frac{1}{2}$ - 3 hours, with occasional stirring. The slurry was allowed to settle for one hour and the supernatant fluid removed with the aid of a water pump. Fresh N. sodium hydroxide was added and the process repeated for a total of 5 times. At the end of this treatment the resin was washed with 5 litres of dis-

tilled water and passed through a 200 mesh sieve. The most efficient way of passing the resin through the sieve was found to be to mix it with about six times its volume of water and blow it on to the sieve from a wash bottle using compressed oxygen at 1Kg/cm². The resin passing through the sieve was used for the columns.

Preparation of the columns. The resin was washed on a Buchner funnel with 1 litre of N. sodium hydroxide using a Schleicher and Schüll filter paper No.595. A paper was placed on top of the resin to prevent channelling. After washing with the sodium hydroxide the resin was washed with 600 ml. of 0.2N pH5 buffer. The resin was then slurried with an amount of 0.2N pH5 buffer such that on settling there was a little more than the same amount of buffer above the resin as there was resin. A suspension of the resin in this buffer was used to pour the columns.

The 150 cm. columns were poured in approximately 6 equal sections. Approximately 50 ml. of the slurried resin was poured at a time using a filter funnel with a drawn out bent tip which touched the side of the chromatography tube. This tended to prevent the inclusion of air bubbles in the column. The resin was packed to a constant height using a pressure of 15 cm. mercury. After the resin had settled to a constant level the supernatant fluid was drawn off using 1/8" tygon tubing attached to a water pump and then another section was poured.

A 250 ml. separating funnel was filled with 0.2N sodium hydroxide containing 0.5% BRIJ 35 and connected to the column. The sodium hydroxide was pushed through the column under 15 cm. pressure until at least 150 ml. had passed through.

The day before use the sodium hydroxide was replaced with pH 3.1 buffer containing 5 ml. BRIJ 35 and 5 ml. thiodiglycol per litre. The buffer was pushed through under 15 cm. pressure until the effluent pH was 3.1, i.e. after about 180-200 ml. of buffer had gone through the column. The column was then ready for use.

The BRIJ 35 was a non-ionic detergent manufactured by the Atlas Powder Co. of U.S.A., and was used to give an even flow through the resin. The thiodiglycol was used to prevent oxidation on the column. It was prepared by distilling "Kromfax" solvent under reduced pressure at a temperature of not more than 180°C.

Operation of columns. The solution to be chromatographed was brought to pH 2.0. It was found that solutions of pH 1.5 could be chromatographed successfully, but more acid solutions caused the flow rate to slow down so much as to be useless. 1 to 6 ml. of sample, depending on the nitrogen concentration, was placed on the top of the column with a bent tipped pipette, after the supernatant pH 3.1 buffer used to equilibrate the column had all been removed or pushed into the column. The test solution was pushed into the column using 15 cm. pressure, and the sides of the tube were washed down with small portions of pH 2.2 buffer such that the total amount of washings did not exceed 1 ml. 5 ml. of pH 2.2 buffer was then placed on the column, and the column mounted over the fraction collector.

A 500 ml. separating funnel containing pH 3.1 0.2N buffer with 5 ml. BRIJ 35 and 5 ml. thiodiglycol was attached to the column and the buffer passed through under 15 cm. pressure.

Water at 30°C was initially circulated through the column jackets. After the emergence of serine, usually at fraction 80, the temperature of the circulating water was increased to 50°C. Moore and Stein (7) recommend an increase of temperature to 75°C after the emergence of histidine in order to separate tryptophan and arginine, but this was not followed as the tryptophan would have been destroyed during the acid hydrolysis of the sample.

At about fraction 145, which is twice the volume corresponding to the aspartic acid peak, the pH and ionic strength of the inflowing buffer was gradually increased to pH 5.1 and 2N using the mixing apparatus described above.

At the end of run the resin was removed from the tube by inverting the tube over a Buchner flask attached to a vacuum pump. The resin was readily removed provided the column was not allowed to dry out. Dry or demi-dry columns were extremely difficult to remove. It was also found advantageous to maintain a column of water above the resin as it was being drawn out of the tubes as this prevented the top of the resin from drying out.

Regeneration of the resin. The resin was regenerated by slurring with three times its volume of distilled water and filtering on a Buchner funnel. The resin was then washed on the funnel with 5 times its volume of N. sodium hydroxide followed by 3 times its volume of 0.2N, pH 5.1 buffer and suspended in twice its volume of 0.2N, pH 5.1 buffer. Best results were obtained if the washings were carried out very slowly, with just sufficient vacuum from a water pump to ensure a steady flow.

Buffers used. The five buffers used were essentially those of Moore and Stein (7) except that it was found that 0.18N, pH 3.1 buffer gave a better separation than the 0.2N, pH 3.1 buffer recommended. These buffers had the following composition:

<u>Buffer</u>	<u>TABLE 2</u>					
	Citric acid H ₂ O g.	Acetic acid glacial g.	Sodium hydroxide pellets g.	Sodium acetate anhydrous g.	Hydrochloric acid 10N ml.	Final volume litres
pH 2.2 ± 0.03* X						
0.2N Na citrate	10.3	-	4.2	-	8.0	0.5
pH 3.1 ± 0.03* X						
0.18N Na citrate	63.3	-	23.4	-	35.4	3.4
pH 5.1 ± 0.02 0X						
2N Na citrate acetate	357.0	73.0	160.0	279.2	-	3.4

TABLE 2 (ctd.)

Buffer	Citric acid H ₂ O g.	Acetic acid glacial g.	Sodium hydroxide pellets g.	Sodium acetate anhydrous g.	Hydro- chloric acid 10N ml.	Final volume litres
pH 5.1						
0.2N Na citrate	acetate		The 2N buffer above was diluted 1/10			
pH 5.51 ⁺ 0.03 ^X						
4N Na acetate	-	100 ml.	-	328.0	-	1.0

* Before use 5 ml. BRIJ 35 solution and 5 ml. thioldiglycol were added for each litre.

O Before use 1 ml. BRIJ 35 solution was added for each litre.

X pH adjustments were made where necessary.

Analysis of fractions. Each tube containing 2 ml. of effluent was collected and placed in order in an aluminium rack made to hold 50 tubes. To each 2 ml. fraction was added from a burette, 1 ml. of ninhydrin reagent (see below). The tubes were capped with aluminium caps and shaken on a Fischer shaker for 30 seconds, and the rack placed in a boiling water bath for 15 minutes. At the end of 15 minutes, the rack was removed from the bath, and to each tube was added 5 ml. of 50:50 ethanol-water as diluent from an automatic burette. The tubes were then shaken for 1 minute and cooled in front of an electric fan. Before being read on a colorimeter at 570 mμ the tubes were wiped with cotton wool. A blank consisting of buffer alone was run with each batch of tubes.

Initially the ninhydrin solution was red. After being added to the effluent fraction the colour changed to yellow and after heating a blue or purplish colour resulted, depending on the amino-acid present. In the absence of any amino-acid or other ninhydrin positive compound the colour reverted to red. Proline and hydroxyproline gave a golden yellow colour. The shaking destroyed any red colour that remained.

The reagent solution was prepared fresh each day and consisted of 2.0 g. ninhydrin and 300 mg. hydrindantin dissolved in 60 ml. of re-distilled methyl cellosolve (ethylene glycol monomethyl ether). 25 ml. of pH 5.51 4N sodium acetate buffer were added, and the solution made to 100 ml. with methyl cellosolve. A red (low actinic) volumetric flask was used.

The ninhydrin used was purchased from Dougherty Chemicals N.Y., U.S.A. and was sufficiently pure not to require recrystallization.

The hydrindantin was prepared in batches as required by dissolving 20 g. ninhydrin in 500 ml. water at 80°C and adding 20 g. ascorbic acid (C.S.R.) dissolved in 100 ml. water at 40°C. Crystallization was almost instantaneous, but was allowed to proceed in a dark cupboard until the solution was cool (35°C) (about 3/4 hour).

The flask was then placed in the deep freeze for half an hour and the hydrindantin filtered off on a sintered glass plate, and washed with ice water until no further colour was leached out. It was dried in a vacuum dessicator over silica gel until completely dry. As with the ninhydrin it was always stored in a dark jar in a dark cupboard.

Hydrolysis of the sample material. The material to be examined was mainly a food with a high carbohydrate content. Hydrolysis was effected by refluxing about 5 g. of sample with 500 ml. of 6N. hydrochloric acid for 23 hours at 120°C. After hydrolysis the hydrochloric acid was boiled off under reduced pressure at 30° - 36°C, using a vacuum pump. The solution was taken down nearly to dryness and the residue taken up in pH 2.2 buffer and made to 50 ml.

Because of the destruction of cystine and methionine during hydrolysis a separate 5 g. sample was treated with 40 ml. of ice cold performic acid (11), made by adding 4 ml. of 30% hydrogen peroxide to 36 ml. of 90% performic acid and allowing the mixture to stand at room temperature for one hour before chilling. The mixture of performic acid and sample was placed in the freezer overnight, and then the excess performic acid reagent removed by distillation under reduced pressure. When most of the reagent had been removed, 500 ml. of 6N hydrochloric acid was added and the whole refluxed for 23 hours at 120°C and treated as outlined above.

Schram et al. (11) recommend chromatography on the sodium chloroacetate form of Dowex 2. However, it was found that a reasonably good separation was obtained on the ordinary Dowex 50 columns if the methionine sulfoxide and methionine sulphone peaks were combined. This is the procedure that was used.

Interpretation of results. Leucine was used as the standard amino acid for colour development and for the construction of a standard curve. The amount of colour produced by 0.1 mM solutions of some twenty two amino acids and of ammonia were compared against that produced by l-leucine. Taking l-leucine as 100, these values are given in the following table.

TABLE 3

THE COLOUR YIELD OF TWENTYFIVE NINHYDRIN POSITIVE COMPOUNDS

<u>Amino Acid</u>	<u>Colour Yield</u>	<u>Amino Acid</u>	<u>Colour Yield</u>	<u>Amino Acid</u>	<u>Colour Yield</u>
Cysteic acid	99	Glycine	95	Histidine	102
Taurine	89	Alanine	97	Arginine	100
Methionine sulfoxide	98	Valine	97	β -Alanine	54
Methionine sulphone	102	Methionine	101	α NH ₂ -n-butyric acid	101
Aspartic acid	94	Isoleucine	100	β NH ₂ -n-butyric acid	101
Threonine	94	Leucine	100	γ NH ₂ -isobutyric acid	100
Serine	94	Tyrosine	100	Ornithine	115
Glutamic acid	99	Pheylalanine	100		
		Ammonia	96		
		Lysine	110		

Two calibration curves were made by plotting the optical density given by varying concentrations of leucine between 0.5 mM and 2.0 mM using 1 ml. and 2 ml. of solution respectively. As 2 ml. fractions were collected during a run the 1 ml. calibrations were not used. From the calibration curve a table (Table 4) was drawn up such that the concentration could be determined directly from the optical density reading between 0.00 and 0.80. With readings above 0.80 the tubes were diluted with 5 ml. of ethanol-water until a reading less than 0.80 was obtained. For reading initially above 1.10 it was found that no reading could be obtained with a dilution of 15 ml., and so 1 ml. was pipetted into a fresh tube and 5 ml. of ethanol-water added, so that the table reading obtained was multiplied by 6 to give the amino-acid concentration.

The concentrations obtained were plotted against the fraction number. This gave a series of peaks which corresponded to each amino-acid and the area under the curve gave the total concentration of that amino-acid. The area under the curve was estimated by adding together the individual tube concentrations that made up the particular curve. The position of each amino-acid was determined by comparison with chromatograms of mixtures of amino-acids of known concentrations. As a double check with the synthetic mixtures, 1 ml. was removed from each tube and after development the tubes corresponding to each peak were combined, desalted by passing through a small column of the acid form of Dowex 2, concentrated and checked by circular paper chromatography (12) against a known amino-acid.

Difficulty was initially experienced because the detergent, BRIJ 35, is non-ionic and was not removed during the desalting procedure. The BRIJ 35 caused the spots to lag and become diffuse. This difficulty was overcome by first soaking the filter paper in a 0.05% solution of BRIJ 35 in water and drying it. Although retardation occurred the spots were sufficiently distinct and separated to allow accurate identification.

The method of Giri (12) was followed fairly closely except that the apparatus consisted of a 12" diameter dessicator fitted with a round piece of wood 12" diameter and 3/8" wide. The paper was pinned to this piece of wood to prevent sagging during the advance of the solvent front. Whatman No.1 papers 25 cm. in diameter, were used and the solvent consisted of the top layer which separated when n-butanol-acetic acid - water in the proportion of 40:10:50 by volume were shaken together.

TABLE 4

CONVERSION TABLE

Concentration in 2 x micromoles per litre

(Leucine Equivalent)

2 cc. sample + 1 cc. ninhydrin + 5 ml. ethanol-water

<u>Reading</u>	<u>Undil.</u>	<u>+ 5 cc.</u>	<u>+ 10 cc.</u>	<u>+ 15 cc.</u>
0.01	2	4	6	8
0.02	5	8	11	14
0.03	8	13	18	23
0.04	10	17	24	31
0.05	13	21	29	37
0.06	15	25	35	45
0.07	18	29	40	51
0.08	21	33	46	59
0.09	23	37	51	65
0.10	26	42	58	74
0.11	28	46	64	82
0.12	31	50	69	88
0.13	33	54	75	96
0.14	36	59	82	105
0.15	39	63	87	112
0.16	41	67	93	119
0.17	44	71	98	125
0.18	46	75	104	133
0.19	49	79	109	139
0.20	51	84	116	148
0.21	54	88	122	156
0.22	57	92	127	162
0.23	59	96	133	170
0.24	62	100	139	178
0.25	64	104	144	184
0.26	67	109	151	193
0.27	69	113	156	199
0.28	72	117	162	207
0.29	74	121	168	215
0.30	77	125	173	221
0.31	80	130	180	230
0.32	82	134	186	238
0.33	83	138	191	244
0.34	88	142	197	252
0.35	90	146	202	258
0.36	93	150	208	266
0.37	95	154	213	272
0.38	98	159	220	281
0.39	100	163	226	289
0.40	103	167	231	295
0.41	106	171	237	303
0.42	108	175	242	309
0.43	111	180	249	318

TABLE 4 (ctd.)

Reading	Undil.	+ 5 cc.	+ 10 cc.	+ 15 cc.
0.44	114	184	255	326
0.45	116	188	260	332
0.46	118	192	266	340
0.47	121	196	271	346
0.48	123	200	277	354
0.49	126	205	284	363
0.50	128	209	289	369
0.51	131	213	295	377
0.52	134	217	301	385
0.53	136	222	307	392
0.54	139	226	313	400
0.55	142	230	319	408
0.56	144	234	324	414
0.57	147	239	331	423
0.58	150	243	336	429
0.59	152	247	342	437
0.60	155	251	348	445
0.61	157	256	354	452
0.62	160	260	360	460
0.63	163	265	367	469
0.64	166	270	374	478
0.65	169	275	381	487
0.66	172	279	386	494
0.67	174	283	392	501
0.68	177	288	399	510
0.69	180	292	404	516
0.70	182	296	410	524
0.71	185	300	416	532
0.72	188	305	422	540
0.73	191	310	429	548
0.74	194	315	436	557
0.75	197	320	443	566
0.76	199	324	449	574
0.77	202	328	454	580
0.78	205	332	460	588
0.79	208	337	467	597
0.80	211	342	473	605

The order in which the amino-acids came off the column was determined and checked by running various mixtures of synthetic amino-acids. The order and position was found to vary slightly with pH, molar concentration of the buffers and the percentage of crosslinking of the resin, Dowex 50. The order of emergence for the pH and molar concentration employed using 8% crosslinked resin is given in Table 5.

Initially 4% crosslinked resin was used but it was found that the rate of emergence of the amino-acids was too rapid, with a subsequent running together of several of the peaks. Adjustments were made to

the pH and buffer concentrations without very much success. Varying mixtures of 4% and 5% crosslinked resins were used with more success, but eventually it was found that the best results were given using 8% crosslinked resin. Almost complete separations were achieved for all the amino-acids, except isoleucine-leucine and to a lesser extent threonine and serine.

TABLE 5

Order of emergence of amino-acids using 8% crosslinked resins

1. Cysteic acid	14. Methionine
2. Hydroxyproline	15. Iso-leucine
3. Methionine sulphoxide	16. Leucine
4. Methionine sulphone	17. β -Alanine
5. Aspartic acid	18. Tyrosine
6. Threonine	19. Phenylalanine
7. Serine	20. γ -amino-n-butyric acid
8. Glutamic acid	21. Lysine
9. Glycine	22. Ammonia
10. Alanine	23. Histidine
11. α -amino-n-butyric acid	24. Tryptophon
12. Valine	25. Arginine
13. Cystine	

4. VITAMINS

Carotene, thiamine, niacine and ascorbic acid were the only vitamins estimated and the methods used are outlined below.

- (a) Carotene. Carotene was determined by the chromatographic method as published in "Methods of Vitamin Assay" (13). The eluted bands were measured in the range 400-500 mu on a "Unicam" SP500 spectrophotometer and the results expressed as β -carotene, although α -carotene and neo-isomers of β -carotene may have been present.

Instead of the magnesium oxide-Hyflo super-cel and the solvent recommended, activated alumina type H was used and found to give good results. The elutant consisted of 3% acetone in 65°-70° C petroleum ether. 60-80 petroleum ether was distilled and the fraction boiling between 65°-72° was used. This amounted to approximately 25% of the petroleum ether distilled.

Initially carotene supplied by Light and Co. (England) was used as a standard but the calibration curves constructed with this material were impossible as it was soluble in methanol whereas pure β -carotene is insoluble in methanol. Eventually Eastman-Kodak carotene containing 20% α and 80% β -carotene, was used and was found to give a good calibration curve.

- (b) Thiamine. The thiochrome method as modified by the laboratories of Krait Foods Ltd. of Melbourne, was used for the thiamine determinations.

This modified method is as follows:

Digestion: Digest the sample with 0.1N sulphuric acid either in a boiling water bath for $\frac{1}{2}$ hour, with shaking or autoclave for $\frac{1}{4}$ hour at 15 lb./sq. inch. Adjust the pH to 4.6 with sodium acetate and incubate overnight at 37° C with takadiastase. Centrifuge and wash twice. If there is a big layer of solids two washings may not be sufficient. Make up to volume and pipette a known amount on to a column of Decalso Y. This should take about 10 minutes to drip through at one drop per second. The longer time it is adsorbed on the column, the harder it is to elute. Elute with acid potassium chloride. Make up to a known volume. Treat the standard similarly. Use a column only once, then regenerate it.

Assay: Weigh out the sample into a beaker.

Add 2 ml. water and 1 ml. concentrated hydrochloric acid. Transfer to a 25 ml. graduated cylinder, washing the beaker with 2 lots of 3 ml. water to give a total volume of no more than 12 ml.

Add about 10 ml. water saturated butanol. Shake for 1 minute horizontally. Either centrifuge or let stand to separate. The top layer should be yellow.

Discard the butanol layer by pipetting it from the top.

Make up to 100 ml. with water in a volumetric flask. Take four 25 ml. graduated flasks. Add 5 ml. alkaline methanol to all the flasks (from a burette). Place 1 drop 2% potassium ferricyanide in flasks 2 and 4. Shake. After one minute add 10 ml. n-butanol (from a burette) and shake all the flasks vigorously for 20 seconds at least. Let stand and settle properly so that no bubbles are left. Pipette off 10 ml. with a wide end pipette, or 9 ml. if this is too difficult, into cells containing $1\frac{1}{2}$ ml. ethyl alcohol. Mix by transferring once to another tube.

Use quinine sulphate (in N/10 sulphuric acid) as standard of a strength such as to give a good scale deflection (0.9-0.18 γ /ml).

The solutions are read using a spectrophotometer with a fluorimeter attachment, as on a fluorimeter.

Standard thiamine: 0.18 γ /ml. thiamine (1 ml. concentrated hydrochloric acid/100 ml.).

Calculation:
$$\frac{F2 - F1}{F4 - F3} \times 0.1 \times 100 \times 10$$

Solution: Alkaline methanol - 300 ml. 10% sodium hydroxide + 200 ml. methanol. Decalso: Mix 100 g. Decalso in a Fuchner funnel with 250 ml. hot 3% acetic acid. Leave for 15 minutes. Drain off using a mild vacuum (on a water pump). Repeat. Wash once with 250 ml. hot 25% potassium chloride. Wash with 250 ml. hot 3% acetic acid. Wash out the potassium chloride with 500 ml. portions of hot water applying mild vacuum continually until free of chloride. Dry below 100° C. For daily use store a moistened portion under water. Use

1 g. per assay.

All solutions must be distilled in an all glass apparatus and no rubber corks should be used except for the alkaline methanol solution.

- (c) Riboflavin. Attempts were made to determine riboflavin fluorometrically, but it was found that the wrong set of filters had been supplied with the spectrophotometer, and although the correct set was ordered immediately, they had not arrived when the laboratory ceased functioning.
- (d) Niacin. The chemical method recommended by the "Methods of Vitamin Assay" (13) was generally followed. However, because of the small amounts of niacin present in most of the plants tested the method was changed so that 10 g. of sample was hydrolyzed with 15 ml. 10N HCl 45 ml. water for 8 hours, filtered and all of the filtrate used for adsorption on Lloyd's reagent.

The microbiological method for niacin determination is much superior to the chemical method as it is much more sensitive and is not as dangerous (cyanogen bromide is used in the chemical method). However, the laboratory was not equipped to handle microbiological assays, and so the chemical method was used.

- (e) Ascorbic acid. Two methods of estimating ascorbic acid were used. The 2:6 dichlorophenol indophenol visual titration method was used for colourless solutions or for solutions where end-point colour change was not masked. For coloured solutions the 4-methoxy-2-nitro aniline method of Schmall et al. (14) as supplied by the Eastman Chemical Company was used. The method is as follows:

Reagents: Amino Reagent. Five hundred mg. of 4-methoxy-2-nitro-aniline are dissolved in 125 ml. of glacial acetic acid and diluted to 250 ml. with 10% sulphuric acid. The reagent is stable for at least two months.

Nitrite Reagent. 0.2% solution of sodium nitrite in water, Ethyl Alcohol or iso-propyl alcohol.

Sodium Hydroxide 10%.

Oxalic Acid Reagent. 0.5% oxalic acid in water.

Ascorbic Acid Standard. Pure ascorbic acid is dissolved in sufficient 0.5% aqueous oxalic acid to give a final concentration of 0.5 mg. of ascorbic acid per ml. (prepared fresh daily).

Procedure: Sample solution. Two ml. of the amino reagent are pipetted into a 200 ml. volumetric flask, followed by 2 ml. of nitrite reagent. The solution is swirled until the orange colour of the amino reagent disappears, 75 ml. of alcohol added, and the contents of the flask mixed well. A 5 ml. aliquot of sample, diluted to contain approximately 0.5 to 2 mg. of ascorbic acid in a 0.5% solution of aqueous oxalic acid is added; the solution is mixed and made alkaline with 25 ml.

of 10% sodium hydroxide. The volume is brought to the mark with water, and the contents again are well mixed. The blue colour developed may be read after 1 minute without the necessity of accurate timing, as it reaches its peak within less than a minute.

Standard solutions: From the ascorbic acid standard, two standard solutions are prepared: Solution A containing 1 mg. of ascorbic acid and Solution B containing 1.5 mg. of ascorbic acid. Both solutions are treated in the same manner as the sample solution.

Reagent Blank: The reagent blank is prepared in the same manner as the sample solution, but with omission of the sample.

Sample Blank: In case of coloured sample solutions a sample blank is prepared, containing the same amount of sample aliquot and all reagents with the exception of the amino reagent.

Colorimetry: The photometer, at a wave length of 570 mμ is set as zero absorbency with the reagent blank in all cases where a sample blank is not necessary. In case of coloured sample solutions, the instrument is set at zero absorbency with water, and the sum of sample- and reagent-blank reading is subtracted from the reading of the sample solution, while only the reading of the reagent blank is subtracted from the readings of the standard solution.

Calculation:

$$A + \frac{(B-A)(E-C)}{D-C} = \text{mg. of ascorbic acid in sample aliquot}$$

Where

A = mg. of ascorbic acid in Standard A
B = mg. of ascorbic acid in Standard B
C = reading (absorbency) of Standard A
D = reading (absorbency) of Standard B
E = reading (absorbency) of sample.

IV. SUMMARY OF RESULTS

The results obtained are tabulated below and will be discussed in the following section.

1: SAMPLES

Food samples were received from many districts and territories. The various samples received, and their source, are listed in the accompanying table 1.

TABLE I

Origin of Food Samples

<u>Latin Name</u>	<u>Common Name</u>	<u>Origin</u>
Artocarpus altilis	Breadfruit fresh smoked	Apia, Western Samoa Reef Is. British Solomon Is. Protectorate
Bambusa sp.	Bamboo shoots	Mandum, Netherlands New Guinea
Bruguiera eriopetala	Aibon Mangrove fruit preparation	Biak, " " "
Cajanus pseudocajan	Pigeon Pea	Oundjo, New Caledonia
Carica papaya	Pawpaw fruit green & ripe Leaves	Noumea, " " (Muting, Netherlands New Guinea (Noumea, New Caledonia
Cocos nucifera	Coconut palm sap mature meat green meat water "apple"	Diak, Netherlands New Guinea (((Noumea, New Caledonia (
Colocasia antiquorum	Taro	Noumea, " "
Colocasia esculenta	Taro tubers leaves	Tie & Noumea, New Caledonia
Cucurbita pepo	Pumpkin leaves	Gailala, Papua
Cyrtosperma chamissonis	Taro	Santo, New Hebrides
Damaropsis kingiana	"leaves" (wild fig family)	Gailala, Papua

<u>Latin Name</u>	<u>Common Name</u>	<u>Origin</u>
Dioscorea alata	Yam white red	Various districts New Caledonia Mare, Loyalty Is.
Dioscorea pentaphylla	Yam green purple	Ateu, New Caledonia Ateu, " "
Eugenia jambos	Jamboe, Eugenia fruit	Netherlands New Guinea
Ficus racemigera	Fig	Ateu, New Caledonia
Gnetum Gnemon	Gnemon leaves	Merauke, Netherlands New Guinea
Hibiscus manihot	Hibiscus leaves	Tie, New Caledonia
Inocarpus edulis	Polynesian chestnut	Apia, Western Samoa Sigatoka, Fiji
Ipomoea batatas	Sweet potato yellow white	Noumea, New Caledonia (Noumea, " " (Wessel Lakes, Netherlands New Guinea
Lumnitzera racemosa	Mangrove leaves	Oundjo, New Caledonia
Manihot dulcis	"Sweet" cassava tubers leaves	Noumea, " " Noumea, " "
Metroxylon sagu	Sago and sago preparations Sago boengkoos Sago bulu Sago ega Sago lempeng Sago senole Sago sep Raw Sago	Muting, Netherlands New Guinea Muting, " " Biak, " " Mandum, " " Biak, " " Biak, " " Mandum, " "
Musa paradisiaca var sapientum oleracea banana rhizome		Lifou, Loyalty Is.
Nipa fruticans	Nipa palm sap	Maropen, Netherlands New Guinea
Oryza sativa	Rice Imported & Fijian rices	Suva, Fiji
Pandanus julianetti	Pandanus "nuts" smoked	Goilala, Papua Marita, "
Pandanus sp.	Pandanus	Noumea, New Caledonia
Pandanus sp.	Pandanus	Tarava, Gilbert & Ellice Is.
Pandanus sp.	Pandanus	Colony
Phaseolus lunatus	Bean	Goilala, Papua
Pteris moluccana	Fern root flour	Fredrik Hendrik Is. Netherlands New Guinea
Pueraria thunbergiana	Magnagna, Kudzu	Ateu, New Caledonia

<u>Latin Name</u>	<u>Common Name</u>	<u>Origin</u>
Sesbania grandiflora	Sesban leaves flowers	{ Noumea, New Caledonia
Solanum sp.	Tuber	Goilala, Papua
Tacca leonpetaloides	Polynesian arrowroot	Tuamotu, French Oceania Is.
Tetragonia expansa	New Zealand spinach	Kone, New Caledonia
Wedelia biflora	leaves	Tie, " "
Xanthosoma sagittifolium	Taro	Noumea, New Caledonia
?	Fungus	Goilala, Papua
?	Dried Fish	Malaita, British Solomon Is. Protectorate

2. PROXIMATE COMPOSITION

The mean results obtained for the water, ash, fibre, ether extract, total nitrogen, soluble reducing sugars and starch is given in the accompanying table 2. in per cent of Edible Portion (E.P.).

TABLE 2

<u>Proximate composition</u>							
	<u>Water</u>	<u>Ash</u>	<u>Fibre</u>	<u>Ether</u>	<u>Total</u>	<u>Reducing</u>	<u>Starch</u>
	%	%	%	Extract	Nitrogen	Sugars	%
(a) <u>Roots and tubers</u>				%	%	%	%
Colocasia antiquorum	59.0	1.3	1.0	0.5	0.120	0.2	35.0
Colocasia esculenta	53.5	0.9	3.0	0.5	0.230	0.5	35.5
Cyrtosperma chamissonis	60.0	1.0	1.0	0.5	0.155	0.5	33.0
Dioscorea alata							
white	54.5	0.5	0.5	0.1	0.270	0.2	38.0
red	68.0	0.4	0.6	0.1	0.344	0.1	30.5
Dioscorea pentaphylla							
green	83.0	0.2	0.7	2.0	0.230	nil	14.0
purple	75.0	0.1	0.7	4.0	0.160	1.5	16.5
Ipomoea batatas							
white	68.0	0.7	0.6	0.1	0.130	0.5	28.0
yellow	68.0	0.9	0.8	0.1	0.100	0.5	29.5
Manihot dulcis tubers	60.0	0.6	1.0	0.7	0.060	8.0	31.0
Musa paradisiaca							
var. sapientum							
oleracea (corm)	70.5	0.4	1.0	0.5	0.180	10.0	18.5
Pueraria thunbergiana	63.0	1.5	5.2	2.2	0.215	6.0	21.5
Solanum sp.	79.0	0.9	2.4	6.0	0.510	nil	5.0
Xanthosoma							
sagittifolium	59.0	1.5	1.0	0.5	0.075	0.2	34.5

	<u>Water</u>	<u>Ash</u>	<u>Fibre</u>	<u>Ether</u> <u>Extract</u>	<u>Total</u> <u>Nitrogen</u>	<u>Reducing</u> <u>Sugars</u>	<u>Starch</u>
	%	%	%	%	%	%	%
(b) <u>Other starchy foods</u>							
Artocarpus altilis							
fresh	80.0	0.3	1.2	1.5	0.150	10.5	13.5
smoked	8.0	4.0	4.5	2.0	0.680	nil	72.0
Bruguiera eriopetala	72.0	3.0	4.0	0.5	0.402	nil	16.0
Metroxylon sagu							
Raw sago	27.0	0.1	0.3	nil	0.035	nil	71.0
Sago boengkoes	42.0	0.5	0.5	2.0	trace	0.2	53.0
Sago bulu	55.0	1.5	1.0	1.0	trace	1.0	39.0
Sago ega	37.5	0.5	1.8	nil	0.010	0.5	53.0
" "	52.5	0.5	0.6	nil	trace	0.5	40.0
Sago lempeng	12.0	0.3	0.2	nil	trace	nil	84.0
Sago senole	23.0	0.5	1.0	16.5	0.300	0.5	59.0
Sago sep	16.5	0.5	1.5	nil	0.098	nil	79.5
Pteris moluccana	13.0	1.0	10.0	nil	2.000	0.2	67.0
Tacca leontopetaloides	18.0	-	0.05	3.0	0.010	nil	76.0
(c) <u>Fruits</u>							
Pandanus sp.							
Gilbert Is.	70.0	0.5	1.0	2.5	0.980	7.2	13.0
New Guinea	67.0	0.5	12.6	14.0	0.590	nil	6.7
Pandanus julianetti	4.0	2.5	22.0	59.0	1.720	2.3	3.5
Carica papaya							
green	91.0	0.5	0.5	0.1	0.128	2.5	3.5
ripe	88.0	0.1	0.5	0.1	0.095	5.5	2.5
Eugenia jambos	88.5	0.01	0.7	5.0	0.032	2.0	0.5
Ficus racemigera	72.0	1.9	7.0	3.2	0.355	4.6	5.7
(d) <u>Nuts</u>							
Cocos nucifera							
mature meat	50.5	1.0	4.2	30.8	0.641	nil	12.0
green meat	56.2	0.8	2.5	27.8	0.721	4.6	4.2
"apple"	87.0	1.1	1.2	2.7	0.173	6.4	0.5
Inocarpus edulis	43.0	1.5	2.5	4.5	0.725	9.5	31.0
(e) <u>Leaves</u>							
Bamboo sprouts	79.0	0.3	3.0	3.0	0.420	3.0	1.5
Carica papaya	75.0	2.5	2.0	2.0	1.255	2.5	6.0
Colocasia esculenta	85.0	2.0	2.3	2.5	0.802	2.8	1.5
Cucurbita pepo	83.5	1.0	1.2	1.0	0.301	10.5	1.0
Dioscoreopsis kingiana	72.4	1.1	5.0	3.5	0.385	5.4	3.0
Gnetum gnemon	93.0	0.1	0.5	0.4	0.211	nil	4.0
Hibiscus manihot	81.0	0.5	4.0	10.0	0.421	nil	0.5
Lumnitzera racemosa	88.5	0.3	3.0	4.0	0.273	0.1	0.1
Manihot dulcis	82.0	1.7	2.4	1.9	1.150	1.2	2.5
Sesbania grandiflora							
leaves	72.7	-	-	-	0.470	-	-
flowers	88.1	0.4	1.5	3.0	0.223	2.7	1.0

	<u>Water</u>	<u>Ash</u>	<u>Fibre</u>	<u>Ether</u> <u>Extract</u>	<u>Total</u> <u>Nitrogen</u>	<u>Reducing</u> <u>Sugars</u>	<u>Starch</u>
	%	%	%	%	%	%	%
Tetragonia expansa	88.5	0.5	1.0	0.5	0.685	1.5	5.0
Wedelia biflora	89.0	0.5	4.0	1.0	0.483	nil	0.5
(f) <u>Saps and juices</u>							
Nipa fruticans sap	81.0	-	-	-	-	10.0	nil
Cocos nucifera							
sap	82.0	-	-	-	-	14.0	nil
water	95.0	0.5	-	0.3	0.093	2.5	nil
(g) <u>Others</u>							
Cajanus pseudocajan	10.0	2.5	8.0	2.5	4.140	0.1	39.7
Dried fish	7.5	1.5	-	7.0	8.854	6.3	1.0
"Fungus"	85.0	trace	0.3	3.5	0.392	3.5	0.5
Oriza sativa							
Indian white	11.0	0.2	0.2	3.6	0.771	nil	66.5
Thailand "	11.9	0.3	0.2	1.7	0.732	nil	73.0
Fiji "	11.4	0.4	0.2	2.0	0.772	nil	70.5
Fiji padi	10.6	-	-	1.7	0.914	nil	48.5
Phaseolus lunatus	70.0	0.5	2.1	0.8	0.742	17.0	8.7

3. Amino Acids

The amino acid composition of the coconut, sweet potato, taro and yam are given in tables 3 - 6 respectively.

TABLE 3

AMINO ACID COMPOSITION OF MATURE MEAT OF COCOS NUCIFERA (COCONUT)

Total nitrogen content 643 mg. per 100 g. fresh sample
48.99 mg. fresh material on column equivalent to 0.315 mg.
total nitrogen

<u>Amino Acid</u>	<u>γ Amino acid</u> <u>found</u>	<u>mg. Amino Acid</u> <u>per 100 g. sample</u>	<u>N. per cent</u> <u>total N</u>
Alanine	113.0	232.8	5.6
γ-amino-n-butyric acid	27.9	57.0	1.2
Arginine	98.3	200.7	9.9
Aspartic acid	203.1	414.4	9.8
Cystine	41.6	84.9	1.6
Glutamic acid	246.2	502.3	6.0
Glycine	66.2	135.2	3.9
Histidine	58.9	120.2	5.1
Leucine	90.5	184.7	3.2
Iso-leucine	54.2	110.6	1.8
Lysine	51.2	104.5	3.1
carried forward	1051.1	2147.3	51.2

<u>Amino acid</u>	<u>γ Amino acid</u> <u>found</u>	<u>mg. Amino Acid</u> <u>per 100 g. sample</u>	<u>N. per cent</u> <u>total N</u>
brought forward	1051.1	2147.3	51.2
Methionine	52.8	107.8	1.5
Phenylalanine	75.3	153.7	2.0
Serine	155.0	316.3	6.3
Threonine	94.6	193.1	3.5
Tyrosine	52.0	106.2	1.3
Valine	100.8	205.5	3.8
Ammonia	31.0	63.3	8.1
	1612.6	3293.2	77.7
Proline	++		
Hydroxyproline	?		

TABLE 4

AMINO ACID COMPOSITION OF IPOMOEA BATATAS
(SWEET POTATO, YELLOW VARIETY)

Total nitrogen content 101 mg. per 100 g. fresh sample
493.1 mg. fresh sample hydrolyzate on column equivalent
to 0.4980 mg. total nitrogen

<u>Amino Acid</u>	<u>γ Amino Acid</u> <u>found</u>	<u>mg. Amino Acid</u> <u>per 100 g. sample</u>	<u>N. per cent</u> <u>total N</u>
Alanine	192.0	39.0	6.0
β-alanine	15.5	3.1	0.5
Arginine	171.4	35.2	10.8
Aspartic acid	280.0	56.8	8.5
Cystine	24.3	4.9	6.0
Glutamic Acid	408.0	82.8	6.2
Glycine	151.5	30.7	5.6
Histidine	79.8	15.8	4.3
Leucine	236.0	48.0	5.0
Iso-leucine	152.2	31.0	3.2
Lysine	176.2	35.8	6.7
Methionine	60.0	12.2	1.1
Phenylalanine	182.5	37.0	3.0
Serine	210.7	44.7	5.6
Threonine	153.0	31.0	3.5
Tyrosine	152.0	30.9	2.3
Valine	145.4	29.8	3.5
Ammonia	50.1	10.1	8.2
	2840.6	578.8	90.0
Proline	+++		
Hydroxyproline	++		

TABLE 5

AMINO ACID COMPOSITION OF COLOCASIA ESCULENTA
(TARO, RED VARIETY)

Total nitrogen 210 mg. per 100 g. fresh sample
135.7 mg. fresh sample hydrolyzate on column equivalent
to 0.285 mg. total nitrogen

<u>Amino Acid</u>	<u>γ Amino Acid</u> <u>found</u>	<u>mg. Amino Acid</u> <u>per 100 g. sample</u>	<u>N. per cent</u> <u>total N</u>
Alanine	121.3	89.4	6.7
β-alanine	118.3	87.2	6.5
α-amino-n-butyric acid	111.6	82.2	5.3
Arginine	113.4	83.5	12.6
Aspartic Acid	14.8	10.9	0.8
Cystine	41.1	30.3	1.7
Glutamic Acid	21.5	15.9	0.6
Glycine	105.5	77.9	6.9
Histidine	32.7	24.1	3.1
Leucine	46.0	34.0	2.9
Iso-leucine	10.1	7.4	0.4
Lysine	123.9	91.2	8.4
Methionine	91.6	67.6	3.0
Phenylalanine	70.1	51.7	2.1
Serine	117.4	86.5	5.5
Threonine	244.4	180.0	10.0
Tyrosine	53.0	39.2	1.5
Valine	102.8	75.2	4.3
Ammonia	34.5	25.5	10.0
	<u>1574.0</u>	<u>1159.7</u>	<u>92.3</u>
Proline	++		
Hydroxyproline	-?		

TABLE 6

AMINO ACID COMPOSITION OF DIOSCOREA ALATA
(YAM, WHITE VARIETY)

Total nitrogen 268 mg. per 100 g. fresh sample
151.6 mg. of fresh sample hydrolyzate on column equivalent
to 0.4062 mg. total nitrogen

<u>Amino Acid</u>	<u>γ Amino Acid</u> <u>found</u>	<u>mg. Amino Acid</u> <u>per 100 g. sample</u>	<u>N. per cent</u> <u>total N</u>
Alanine	111.5	73.6	4.3
β-alanine	9.1	6.0	0.4
carried forward	120.6	79.6	4.7

<u>Amino Acid</u>	<u>γ Amino Acid</u> <u>found</u>	<u>mg. Amino Acid</u> <u>per 100 g. sample</u>	<u>N. per cent</u> <u>total N</u>
brought forward	120.6	79.6	4.7
γ-amino-n-butyric Acid	23.9	15.8	0.8
Arginine	193.4	127.5	15.1
Aspartic Acid	239.7	158.0	9.0
Cystine	21.9	14.5	0.6
Glutamic Acid	326.5	215.0	6.1
Glycine	81.5	53.8	1.7
Histidine	53.2	35.1	3.6
Leucine	134.5	88.8	3.5
Iso-leucine	86.1	56.8	2.2
Lysine	89.0	58.8	4.2
Methionine	21.9	14.5	0.5
Phenylalanine	121.5	80.2	2.5
Serine	152.0	100.0	5.1
Threonine	85.0	56.0	2.5
Tyrosine	83.3	55.0	1.6
Valine	116.5	77.0	3.3
Ammonia	58.6	35.9	11.9
	2009.1	1322.3	78.9
Proline	++		
Hydroxyproline	++		

4. VITAMINS

The mean results obtained for the various vitamin analyses are given in the following table.

TABLE 7
VITAMIN CONTENT OF SELECTED FOODS

	<u>Carotene</u> mg%	<u>Thiamine</u> mg%	<u>Niacin</u> mg%	<u>Ascorbic</u> <u>Acid</u> mg%
<u>Roots and tubers</u>				
Colocasia esculenta	0.003	0.270	0.202	5
Cyrtosperma chamissonis	0.008	0.319	0.059	3
Dioscorea alata				
red	0.132	0.125	0.297	4 - 8
white	nil	0.145	0.191	trace - 8
Dioscorea pentaphylla				
green	0.556	0.061	-	trace
purple	0.720	0.048	0.099	-
Ipomoea batatas				
yellow	0.149	0.077	0.242	68
white	0.036	0.065	0.700	62

	<u>Carotene</u>	<u>Thiamine</u>	<u>Niacin</u>	<u>Ascorbic</u> <u>Acid</u>
	mg%	mg%	mg%	mg%
Manihot dulcis tubers	nil	0.038	0.279	26
Musa paradisiaca var.				
sapiantum oleracea (corm)	0.064	0.028	0.198	trace
Pueraria thunbergiana	nil	-	0.243	1
Xanthosoma sagittifolium	trace	0.258	0.329	6
<u>Other starchy foods</u>				
Artocarpus altilis	-	0.080	-	72
<u>Fruits</u>				
Ficus racemigera	0.975	0.079	0.401	2
Pandanus sp.				
Gilbert Is.	0.011	0.034	-	5
New Caledonia	-	-	-	8 - 18
New Guinea	-	0.140	1.372	-
Pandanus julianetti (smoked)	-	0.225	1.310	-
<u>Nuts</u>				
Cocos nucifera				
"apple"	-	-	-	4
meat mature	-	0.085	-	4
water	-	nil	-	3
Inocarpus edulis	nil	0.261	-	2
<u>Leaves</u>				
Butan kap	0.046	-	-	-
Dammaropsis kingiana	1.459	0.128	0.465	-
Manihot dulcis	12.370	0.335	1.068	275
Pumpkin leaves	2.500	0.122	0.750	-
Sesbania grandiflora	11.200	0.263	0.634	233
flowers	0.151	0.102	0.425	68
Solanum sp.	3.990	0.126	0.747	-
Tetragonia expansa	1.494	-	-	28
Wedelia biflora	-	-	-	10
<u>Others</u>				
Phaseolus lunatus	-	0.222	1.293	-
Fungus	-	0.016	2.003	-
Oryza sativa				
white imported from India	-	0.055	1.690	-
" " " Thailand	-	0.079	1.340	-
" grown & milled in Fiji	-	0.075	1.570	-
Padi	-	0.266	3.720	-
Cajanus pseudocajan	-	0.006	-	21

5. NEW HEBRIDEAN HUMAN MILK

Fiftyone samples of milk from New Hebridean mothers were examined for their lactose, protein, fat, ash, calcium and phosphorus contents. Results are given in the following table.

TABLE 8

MEAN RESULTS OF THE MAJOR CONSTITUENTS OF NEW HEBRIDEAN MOTHERS MILK SAMPLES

<u>Lactation period</u>	<u>No. of samples</u>	<u>Lactose</u>	<u>Protein</u>	<u>Fat</u>	<u>Ash</u>	<u>Calcium</u>	<u>Phosphorus</u>
2 - 5 months	(Mean 18 (Range (S.D.	5.05 4.2-5.8 0.453	1.17 0.86-1.42 0.141	3.8 1.8-6.4 1.71	188 170-210 13.03	27.4 20.8-34.0 4.14	15.9 13-18 1.61
6 - 11 months	(Mean 15 (Range (S.D.	5.00 4.4-6.0 0.519	1.09 0.86-1.26 0.141	3.80 1.8-6.0 1.36	178 165-205 13.31	25.1 19.2-34.0 4.92	15.1 12-19 2.39
12 - 24 months	(Mean 18 (Range (S.D.	4.91 4.1-5.4 0.466	1.05 0.81-1.26 0.118	3.80 2.0-6.2 1.35	176 155-210 17.18	24.9 18.6-33.8 4.89	14.7 12-19 2.11
2 - 24 months	(Mean 51 (Range (S.D.	5.00 4.1-6.0 0.432	1.10 0.81-1.42 0.140	3.80 1.8-6.4 1.53	181 155-210 16.37	25.8 18.6-34.0 4.71	15.2 12-19 2.06
2 - 7 months	(Mean 7 (Range (S.D.	6.35 5.2-7.4 1.517	0.96 0.82-1.10 0.99	4.1 2.6-7.1 1.54	219 210-240 13.64	31.1 26.5-34.5 3.14	15.6 13-18 1.92
Canberra Mothers							

Comparison between New Hebridean and European milks is shown in table 9.

TABLE 9

COMPARISON BETWEEN NEW HEBRIDEAN AND EUROPEAN MILKS

		<u>Lactose</u>	<u>Protein</u>	<u>Fat</u>	<u>Ash</u>	<u>Calcium</u>	<u>Phosphorus</u>
New Hebrides	Mean	5.00	1.10	3.8	181	25.8	15.6
	Range	4.1-6.0	0.81-1.42	1.8-6.4	155-210	19-34	12-19
N.R.C. Bulletin (7)	Mean	7.00	1.10	3.8	210	34.0	16
	Range	4.2-9.2	0.7-2.0	1.0-8.4	160-270	17-61	7-35

There did appear to be a slight decline in the non-fatty constituents over a lactation period of 2 - 24 months. The lactose and calcium contents of the Melanesian milk did appear to be lower than in European milk, but because of the small number of samples examined, it is hard to say whether these differences are significant.

V. DISCUSSION OF RESULTS

1. Roots and Tubers

Taros. *Colocasia antiquorum* and *Colocasia esculenta*, *Xanthosoma sagittifolium* and *Cyrtosperma chamissonis* were examined. In all, some twenty different samplings of tubers drawn mainly from New Caledonia, were studied for their proximate compositions. One sampling only of *Colocasia esculenta*, *Cyrtosperma chamissonis* and *Xanthosoma sagittifolium* tubers were examined for the vitamin content. In all cases, naturally, variations in composition were found, but in no case did this variation exceed 10%, calculated on a dry weight basis, for the constituents determined.

The water content varied over a wider range and was probably dependent on the maturity of the tubers and the length of time they may have been stored.

The variation in water content was :

- *Colocasia antiquorum* 55.0 - 73.2% (10 samplings).

The mean value was 60.2%, but the value most representing a median value was 59% and the results given were based on this value.

- *Colocasia esculenta* 51.0 - 57.0% (4 samplings).

The mean value was 53.5% and the results given were based on this value.

- *Cyrtosperma chamissonis* 60.0% (1 sampling).

- *Xanthosoma sagittifolium* 57.0 - 65.0% (5 samplings).

The mean value was 59.0% and the results given were based on this value.

Four samples of the leaves of *Colocasia esculenta* were also examined for proximate composition.

From the figures obtained it can be said that all taros are poor sources of carotene, ascorbic acid, niacin and fat, but fair sources of thiamine. The *Colocasia* and *Cyrtosperma* are better sources of protein than the *Xanthosoma*, but none of the taros constitute a good protein source. Excepting the *Colocasia antiquorum* and the *Cyrtosperma* the total nitrogen figures are lower than the average figures given in the literature.

The leaves of *Colocasia esculenta*, however, constitute a reasonable protein source, and a mixture of taro tubers and leaves would be expected to give a good balanced diet.

Six samples of *Colocasia esculenta* and *Colocasia antiquorum* were examined for their amino-acid content, but it was very difficult to obtain good hydrolyzates and complete analyses. Only one good complete analysis was obtained although several good partial analyses were obtained. The good analysis for *Colocasia esculenta* is given above.

It will be noticed from the figures that the taro has a very low content of iso-leucine, aspartic acid and glutamic acid. This was observed with all the hydrolyzates examined, and in some cases neither aspartic nor glutamic acids could be found. The arginine and threonine contents are high, the methionine content is very good for a vegetable protein, whilst the content of the other amino-acids is fair to good. β -alanine was found in nearly all the samples examined in approximately the same concentration as for alanine. In all the Colocasia examined a strong and distinct peak was found between alanine and valine corresponding to the position of α -amino-n-butyric acid when a synthetic mixture containing this amino-acid was chromatographed. Circular paper chromatograms were run using material collected from a column and pure synthetic α -amino-n-butyric acid. Both spots had the same Rf value and, when mixed, moved as a single spot. From this evidence it can be concluded that Colocasia taros contain α -amino-n-butyric acid. Proline was also found, but no definite indication of the presence of hydroxyproline was seen.

An unknown peak was observed in several samples, coming just after valine but insufficient material could be obtained to enable any identifications to be made.

Sweet Potatoes. All the sweet potatoes examined were Ipomoea batatas, but quite big differences were found in the colour and texture of the tubers. The colour varied from a dead white to a deep orange. The carotene content was found to be correlated with the depth of colour. This topic has been the subject of much recent work in the United States (Arthur, et al. (15)).

Eighteen samplings were examined for proximate composition, ten "white" varieties and eight "yellow" varieties. There was little or no difference in the proximate composition between the two varieties except that the total nitrogen figure of the white varieties was higher than the yellow varieties both on a dry and fresh weight basis.

The water content of all the samplings fell within the range 66 - 72% and for both varieties the mean was 68%.

The ascorbic acid content of sweet potatoes was considerably higher than all the other roots examined, and fell within the range of published figures. The thiamine content was low but the niacin content was fair. The white variety appeared to have approximately three times as much niacin as the yellow variety, but insufficient analyses were carried out to know whether these figures were significant.

The amino-acid composition of the two varieties, on an amino nitrogen as a per cent of the total nitrogen basis was approximately the same, as is shown in the accompanying table 10.

The sweet potato is only a fair source of histidine, methionine, and cystine. The other amino-acids are present in fair to good quantities. β -alanine was found to make up approximately 6% of the total amino-acids. Both proline and hydroxyproline were found to be present.

Yams. Two species of yams were examined: *Dioscorea alata* and *Dioscorea pentaphylla*. In each species there were two marked varieties based on colour differences. The *Dioscorea alata* varied in colour from white to a purplish red, and the *Dioscorea pentaphylla* from green to a deep purple.

Two samplings of both varieties of *Dioscorea pentaphylla* were examined for proximate composition and twenty-one samplings of *Dioscorea alata*, eight red varieties and thirteen white varieties were examined.

The proximate composition of both varieties of *Dioscorea pentaphylla* was similar on a dry weight basis, although the total nitrogen and water content of the green variety did appear to be higher than the purple variety but, from only two samplings of each it is hard to draw any valid conclusions. The vitamin content of both varieties is low, there being only traces of ascorbic acid and very little thiamine and niacin.

The proximate composition of the red and white varieties of *Dioscorea alata* was the same on a dry weight basis although there was a considerable difference in the water content between both varieties. The water content of the white variety ranged from 51.5 - 58.0%, with a mean value of 54.5%, and the results are based on this mean figure of 54.5%. The water content of the red variety ranged from 62.0 - 73.0% with a mean value of 68.0%, and the results are based on this mean figure of 68.0%.

TABLE 10

COMPARISON OF THE AMINO ACID CONTENT OF YELLOW AND WHITE VARIETIES OF IPOMOEA BATATAS (SWEET POTATO) ON AN AMINO NITROGEN AS A PER CENT OF THE TOTAL NITROGEN BASIS.

<u>Amino Acid</u>	<u>Yellow variety</u>	<u>White variety</u>
Alanine	6.0	6.2
β -alanine	0.5	0.4
Arginine	10.8	10.0
Aspartic Acid	8.5	8.6
Cystine	6.0	6.2
Glutamic Acid	6.2	6.4
Glycine	5.6	5.1
Histidine	4.3	4.3
Leucine	5.0	5.2
Iso-leucine	3.2	3.3
Lysine	6.7	6.9
Methionine	1.1	1.4
Phenylalanine	3.0	3.0
Serine	5.6	5.1
Threonine	3.5	3.5
Tyrosine	2.3	2.5
Valine	3.5	3.5
Ammonia	8.2	10.2
Total nitrogen	101 mg/100 g.	132 mg/100 g.

The vitamin content of *Dioscorea alata* was also low although higher than *Dioscorea pentaphylla*. The ascorbic acid content ranged from a trace to 8mg.%. The thiamine figures were higher than those published for Hawaiian varieties, but the niacin content was only about half the Hawaiian values.

In each case it was found that the red or purple varieties had a higher carotene content than the white or green varieties. The white varieties were found to have at the most only a trace of carotene and in several samples no carotene could be detected. The reddish *Dioscorea alata* were not good sources of carotene by any means, containing only 0.10 - 0.15 mg %. The *Dioscorea pentaphylla* were better carotene sources, having 0.55 - 0.73 mg %. Insufficient samples were examined, however, to be able to draw any firm conclusion on the relevancy of colour to carotene content in yams.

The amino acid composition of both varieties was very similar on the basis of amino acid percentage of total nitrogen, as is shown in the accompanying table 11. The sulphur amino acid content of the yam proteins was low and, excepting for possibly serine and glutamic acid, the content of the other amino acids may be ranked as poor to fair. Proline and hydroxyproline were both present. A small amount of β -alanine was also found together with a small amount of γ -amino-n-butyric acid. This latter amino acid was also found in coconut protein in a higher concentration and its identity was shown using circular paper chromatography.

TABLE 11

COMPARISON OF THE AMINO ACID CONTENT OF RED AND WHITE
VARIETIES OF DIOSCOREA ALATA (YAM) ON AN AMINO
NITROGEN AS A PER CENT OF THE TOTAL NITROGEN BASIS

<u>Amino Acid</u>	<u>White variety</u>	<u>Red variety</u>
Alanine	4.3	4.1
β -alanine	0.4	0.5
γ -amino-n-butyric acid	0.8	1.0
Arginine	15.1	14.8
Aspartic acid	9.0	9.5
Cystine	0.6	0.5
Glutamic acid	6.1	6.8
Glycine	1.7	1.7
Histidine	3.6	3.4
Leucine	3.5	3.4
Iso-leucine	2.2	2.6
Lysine	4.2	4.0
Methionine	0.5	0.6
Phenylalanine	2.5	2.5
Serine	5.1	5.3
Threonine	2.5	2.5
Tyrosine	1.6	1.8
Valine	3.3	3.5
Ammonia	11.9	11.5

Total nitrogen 268 mg/100 g. 346 mg/100 g.

Banana Corm. One sampling of *Musa paradisiaca*, var. *sapientum* ole was obtained. This corm comes from a fruitless variety of banana, which in outward appearance resembles the ordinary banana. The corm resembles a *Xanthosoma* corm in appearance and is prepared and used in a similar manner to taro.

The main difference between this food and the taros, yams and sweet potatoes is its relatively high content of soluble sugars and consequent reduction in starch content. It contains only a trace of ascorbic acid and is low in the other vitamins.

Cassava, Manioc. Both the tubers and leaves of *Manihot dulcis* were examined. *Manihot dulcis* is the "sweet" cassava and may be eaten cooked, similarly to taro, without any intricate preparation, as the glycoside content is much lower than in *Manihot esculenta* or *Manihot utillissima*, the bitter variety found in Africa, Asia and South America, and as a consequence *Manihot dulcis* is not normally toxic.

The results obtained generally agree with other published work. The leaves are an extremely good source of carotene, a fair source of niacin, and a good source of ascorbic acid. The tubers however, are not a good vitamin source, having no carotene and only minor amounts of the other vitamins estimated. The tubers may generally be regarded as composed only of starch and water.

Adriaens (16) has carried out a full examination of African cassava and has shown that the protein present in prepared cassava has a high ornithine content. This high ornithine content is apparently derived from arginine during the fermentation process used to remove the glucosides. No work was carried out in Noumea on the amino acids present in *Manihot dulcis*, and it would be interesting to see whether the proteins from raw *Manihot dulcis* and *Manihot esculenta* were similar, and also whether there is any tendency toward the formation of ornithine in *Manihot dulcis* during its preparation and cooking.

Magnana, Kudzu. Two samples of *Pueraria thunbergiana* tubers were obtained. These were the long roots of a vine and were extremely fibrous. The use of *Pueraria thunbergiana* has declined considerably and it is now mainly used only during food shortages and emergencies. It is an exceedingly poor vitamin source, but it does contain 0.2% total nitrogen. The protein, however, was not examined. Its high fibre content detracts considerably from its food value. It is also difficult to prepare for use.

Solanum sp. One sample of a *Solanum* tuber from Papua was received. This tuber appeared to be a good food. It contained 0.5% nitrogen on an 80% approx. water basis and was a good source of carotene and a fair source of niacin. An ascorbic acid determination was not made.

2. Other Starchy Foods

Breadfruit. Two samples of fresh breadfruit, *Artocarpus altilis*, were received from Western Samoa and a sample of dried breadfruit was received from

the British Solomon Islands.

The main point of interest between the fresh and dried breadfruit was the variation in the calcium content. The calcium content of the dried breadfruit was about three times what would have been expected, and was probably due to contamination by coral dust during the preparation.

The method used for drying the breadfruit was to cook the fresh ripe fruit in the ashes of a fire for an hour. The cooked product was left for a day and then peeled, the seeds removed, and it was cut into small pieces which were arranged on a net to a depth of four or five inches. This net was suspended over a smokeless fire and the pieces were turned continuously for about six hours.

The dried breadfruit may be kept for twelve months or more if stored in coconut baskets on racks above the kitchen fire place.

Aibon. Aibon is a starch paste preparation made from the fruit of a mangrove, *Bruguiera eriopetala*, by the peoples of Netherlands New Guinea. One sample only was received and this was shown to have a relatively high protein content (about 2.5% N x 6.25, on a fresh weight basis). It also had a relatively high ash and fibre content.

Fern root flour. Two samples of a flour prepared from the root of *Pteris moluccana* were received from Netherlands New Guinea.

The flour is prepared by grinding the fern root and extracting the ground material with water and squeezing through "cloth".

The extract was then dried in the sun. The "flour" had an extremely high fibre content, 10%, but also contained about 12% crude protein (N x 6.25).

Sago. Samples of raw sago (*Metroxylon sago*) and of six sago preparations were received from Netherlands New Guinea. The method of preparation of the various sagos is as follows :

Raw sago: The trunk of the sago palm is split and the pith is removed by grating or crushing with a stone adze or sharpened piece of bamboo. The crushed sago is kneaded by hand whilst the sago is being washed, and the starch and water mixture is collected and filtered through a fibrous "cloth". The drained starch is then partially dried and either stored in small palm leaf baskets or further processed.

Sago Boengkoes: The raw sago is made into a thick paste or dough which is drawn out into a rod shape, wrapped in palm leaves and roasted on a fire.

Sago bulu: Hollow bamboos are filled with a raw sago paste and cooked over a fire.

Sago_ega: The sago is pressed into brickettes and wrapped in sago palm leaves.

Sago Lempeng: The raw sago is placed in tins or other receptacles and baked until it is hard and dry.

Sago senole: The raw sago is mixed with grated coconut and baked in the form of a flat cake or biscuit.

Sago sap: This is similar to sago lempeng except that the raw sago is baked into thin flat biscuits on a hot stone or other flat surface.

Excepting for sago senole it can be said that all the sago preparations examined consisted only of starch and water in varying proportions with small amounts of extraneous ash and fibre derived during preparation. Raw sago contains a small quantity, 35 mg%. of total nitrogen, but this seems to be lost during the various preparative procedures.

In all, four different shipments of sago preparations were received, each containing at least two samplings of the sago preparation. As would be expected, the water content varied considerably and more or less average values have been taken. The sago ega samples received, however, fell into two general classes on a water content basis and the results for these two classes are given.

From a nutritional point of view the only preparation which had any value, other than as a source of calories, was sago senole. The addition of grated coconut added nitrogen and fat, both of which are either completely lacking, or present only in trace proportions in the other sago preparations examined.

Polynesian arrowroot. Tacca. One sample of tacca flour (*Tacca leontopetaloides*) was examined. This flour was prepared from the tacca tubers in the Tuamotu Islands by washing the grated tuber twice with sea water, then once with fresh water and drying in the sun. The procedure removed the bitter factor which is present in the tubers.

The flour consisted essentially of starch and water, but did contain 3% ether extractable material.

3. Fruits

Pawpaw. Eight samplings of the ripe fresh fruit of *Carica papaya* and five samplings of the green fruit were examined for proximate composition, together with five samplings of young leaves.

Both green and ripe fruit had a very high water content. On a dry weight basis the total nitrogen values were good.

The leaves contain quite a high protein content.

The figures obtained for leaves and fruit agreed reasonably well with figures that had been previously published.

Eugenia fruit. Two samples of the fruit of *Eugenia jambos* were examined for proximate composition. As a food they may be regarded as being just average and do not possess any outstanding properties.

Fig. A sampling of a New Caledonian native fig, *Ficus racemigera*, was examined. The fruit was small, about the size of a fingernail in diameter, but sweet. The carotene content was good, nearly 1 mg%, but the content of other vitamins was low.

The protein content on a dry weight basis was good although the fibre content was high.

Pandanus. Three samplings of pandanus fruits (*Pandanus* sp.) were received from Tarawa and two prepared samplings from Papua. Two samplings of the roasted nuts of *Pandanus julianetti* were also received from Papua.

The pandanus constitutes an important part of the diet of the atoll peoples of Micronesia and Polynesia.

The pandanus from Tarawa were a deep orange in colour. Miller et al. (18) stated the yellow orange colour was due to carotenoid pigments. However, it was found that the pigment in the Tarawa pandanus was water soluble and gave typical anthocyanin reactions. The β -carotene content was found to be only 0.011 mg% (range 0.009 - 0.013 mg%), which is much below Miller's figures, 0.184 - 1.242 mg%. Unfortunately the New Guinea pandanus samplings had not been prepared for carotene assay and no carotene analysis could be made on them. There would appear to be a lot of work to be done on the carotene content of pandanus and on the non-carotenoid pigments.

The niacin content of the New Guinea pandanus was good. It was found that the Tarawa pandanus contained an extremely active ascorbic acid oxidase. Tests were made on New Caledonian pandanus, which are inedible, but these were found to possess no ascorbic acid oxidase activity.

Pandanus "keys" are extremely fibrous, and the analyses were made on edible portions of the Tarawa pandanus omitting much of the fibre which would normally be discarded after chewing. This would account for the big difference in fibre content between the Tarawa and New Guinea pandanus, as the latter were prepared by chopping up the whole "edible" segments. The total nitrogen content of both species of pandanus was good. The New Guinea pandanus also contained a high ether extractable content.

The *Pandanus julianetti* nuts were prepared by roasting the pandanus keys and then removing the edible nuts. These nuts had a high niacin and a good crude protein content. The amount of ether extractable material was also high. The high fibre content was caused by cellulose strands which adhere closely to the nut, but when consumed, much of this cellulose is discarded after chewing

4. Nuts

Coconut. Although the coconut, *Cocos nucifera*, has been the subject of much work previously, many determinations were made on the nut at various stages of ripening, and for specific constituents.

The values obtained for the meat and water of the nut agree generally with figures which had been published previously.

The coconut "apple", which is the spongy mass which develops inside the coconut when the nut is sprouting was of interest, as on some atolls this is often the only fresh "vegetable" available, and is widely eaten both raw and cooked. The water content was high, but on a dry weight basis it had a high content of protein and soluble sugars.

The amino acid content of the whole coconut was examined and the results are given in table 3. The amino acid make-up was reasonably good and seemed to be well balanced, although it was low in sulphur amino acids. The aspartic and glutamic acid content was good. γ -amino-n-butyric acid was found to be a constituent amino acid. Its identity was checked by circular paper chromatography against pure synthetic γ -amino-n-butyric acid. Two small peaks were found consistently between histidine and arginine but their identity could not be established.

The sap of the coconut palm is very widely used as a drink and is known as "toddy". Toddy is prepared by binding a flower spathe just before it opens and slicing off the end. The sap oozes from the cut end and is allowed to drip into a container and is collected once or twice a day. After each collection the end of the spathe is shaved to expose a fresh surface. The process continues until the spathe has been shaved away completely.

If allowed to stand the toddy rapidly ferments. In some areas only unfermented toddy is drunk, in others only fermented toddy and in still others both forms are consumed.

Polynesian Chestnut. Three samplings of *Inocarpus edulis* were obtained, two from Fiji and one from Western Samoa.

These nuts had a low fat and a high starch content. The protein content was comparable with that of the coconut, and similarly to the coconut, they had a low vitamin content.

5. Leaves

Bamboo shoots. One sample of bamboo shoots, *Bambusa* sp., was received from Netherlands New Guinea. They contained a relatively high protein content, but otherwise were of little interest.

Pumpkin leaves. One sample of the leaves of *Cucurbita pepo* was received from Papua. This was found to be a good source of carotene and protein, and was rich in soluble reducing sugars.

Dammaropsis kingiana, leaves. One sample of *Dammaropsis kingiana* leaves was received from Papua. This was found to be a good source of carotene and protein.

Gnemon leaves. One sample of the leaves of *Gnetum gnemon* from Netherlands New Guinea was examined. This sample showed a very high water content, and unlike the other leaves examined a high starch content, about 60% on a dry weight basis, together with a reasonably high protein content.

Hibiscus leaves. The leaves of *Hibiscus manihot* are widely eaten as a green vegetable and several samplings were obtained in New Caledonia. These showed a good protein content and a high content of ether extractable matter. The water and fibre contents were also high.

Mangrove leaves. The leaves of the mangrove, *Lumnitzera racemosa*, are used by the natives of New Caledonia as an emergency food during periods of food shortage. One sample was examined which showed a high water content together with a high fibre and ether extract content and a reasonable protein content.

Sesban leaves and flowers. *Sesbania grandiflora* is a large tree used in Noumea as an ornamental tree, but in Netherlands New Guinea and French Oceania as a food tree; the leaves and flowers being eaten.

The leaves were found to be an extremely rich source of carotene and a good source of ascorbic acid. The protein content was also found to be good.

The flowers were found to be only a fair source of vitamins and protein.

New Zealand Spinach. New Zealand spinach, *Tetragonia expansa*, is found throughout the Pacific, and on the low atolls is one of the few green vegetables. It grows generally along beaches and in sandy regions. Samples were obtained from New Caledonian beaches. It was found to be a good source of carotene and protein.

Wedelia biflora. *Wedelia biflora* is a plant with succulent leaves, which are used as a cooked green vegetable by the natives in New Caledonia. Two samples were examined. These showed a good protein content, but had a high water and fibre content.

Other results, for the leaves of *Colocasia esculenta* and *Manihot dulcis*, are discussed with the results for the tubers of these plants, and for the leaves of *Carica papaya* with the results for the fruit.

6. Saps

Nipa sap. A toddy is prepared from the palm. *nipa fruticans* similarly to the coconut toddy. One sample of unfermented Nipa toddy from Netherlands New Guinea was examined for its sugar content.

Results for coconut toddy and coconut water are discussed together with the values for coconut.

7. Others

Pigeon Pea. A type of pigeon pea, *Cajanus pseudocajan*, which is eaten both fresh and dry, was examined for its proximate composition. The dry pulse was found to have a high protein and starch content and would constitute a good food, even though the fibre content is slightly high. The thiamine content was very low.

Dried Fish. Fish flakes were prepared in the British Solomon Islands by cleaning the fish and boiling them for 30 minutes in a mixture of 3 parts of sea water and 1 part of coconut water. After cooking, the bones were removed and the flesh was broken into small pieces which were slowly dried for 24 hours over a small fire. The final product consisted of yellow-brown flakes 1/4 to 1/2 inch long which had a pleasant though slightly smoky taste.

These flakes had a high protein content, were very palatable and kept well. They would be a valuable item of trade between coastal and inland peoples, and their high salt content would make them very attractive to inland peoples where salt is a scarce commodity.

A note on this work was submitted to the Fiji Agricultural Journal but reprints have not yet been received.

Fungus. A sample of Fungus from Papua was examined. This was found to be a good source of niacin and a fair source of protein.

Rice. Samples of rice were received from Fiji. These samples were of imported white rices from India and Thailand and a white rice grown and milled in Fiji, and a Fijian padi. The results are tabulated in Tables 2 and 7 and generally agree with the published figures.

Bean. A sample of *Phaseolus lunatus* was received from Papua and examined. This was a good source of niacin and protein and contained a high content of soluble reducing sugar.

VI. WEANING FOOD FROM THE COCONUT

One of the major problems in the South Pacific is the lack of suitable weaning foods. In most areas the cattle population is negligible and as a consequence fresh animal milk is in extremely short supply or unobtainable at all. In the urban areas and on some of the richer islands, imported tinned and dried milks are available, but the cost is high and the utilization is not very efficient.

Because of these factors it was felt that a search should be made for a possible vegetable "milk" that could be readily produced and which would be cheap.

The coconut is found throughout the Pacific, and in many places constitutes both an important part of the diet and an important source of revenue. The revenue is derived from the sale of copra, the dried kernel of the coconut, which is exported to Europe, Australia and the United States for the extraction of coconut oil. As the oil extraction mills are mainly situated in metropolitan countries, far removed from the site of production, the by-products, mainly copra meal, are not available to the local producers.

Copra meal has long been recognised as an excellent cattle food because of its high content of good class protein and it was thought that this protein could be used in the production of an infant food. Work on this problem was accordingly undertaken.

It soon became evident that the copra meal itself would not be a suitable starting material, either from the food or the economic point of view. The press cake is often foul smelling with a decidedly rancid flavour. Also the cost of returning the final product, if any, to the Pacific would be as high if not higher than the cost of skim or whole powdered cows' milk. Efforts were therefore made to develop a process of oil extraction which would be simple and economical to establish and operate. The export of oil would thus replace the export of the copra and would leave the protein in the area of production.

In 1937 a method of extracting oil from fresh coconut by means of shredding water extraction and subsequent separation of the oil from the protein and carbohydrate portions was patented by a Filipino, V.G. Lava (19). This method differed fundamentally from the copra-oil process in that the nut protein was said to become available as a palatable food for human consumption, and the oil was of a very high grade of purity. A pilot plant was established in the Philippines and operated until 1941. Lava died during the war and the work stopped.

The process briefly consisted of:

- (a) husking and shelling the nuts
- (b) slicing the meat and washing the sliced meat

- (c) mixing the sliced meat with water at 22° - 90° C
- (d) pressing
- (e) repeating (c) and (d) twice
- (f) centrifuging the oil-water emulsion to separate the oil.

The products obtained from this process were:

- (a) a coconut oil
- (b) a press cake
- (c) a protein concentrate
- (d) an aqueous sugar-salt solution.

A full investigation of this process was made and several modifications were introduced. It was found that a much better extraction of protein could be obtained if 1.5% sodium chloride, i.e., 50:50 sea water : fresh water, was used instead of fresh water, and that the oil-water emulsion could be broken and separated using a dairy cream separator.

The products obtained using the modified process were:

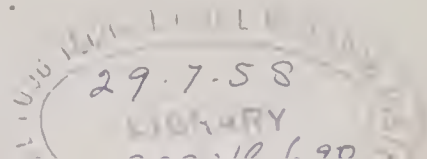
- (a) coconut oil of a very high quality with a very low content of free fatty acids;
- (b) a press cake which when dried contained 7 - 9% protein (N x 6.25), 2% oil and a large quantity of fibre;
- (c) a protein concentrate which contained 5 - 7% water, 75 - 85% protein (N x 6.25), 1 - 2% ash. This protein had a bland taste but mixed readily with coconut water, soups or dilute saline;
- (d) an aqueous sugar-mineral solution which contained about 5% total solids and up to 1% protein (N x 6.25).

The protein concentrate was the material of most interest, and approximately 80% of the total nitrogen of the fresh nut was found in this concentrate. The fact that this protein mixed readily with coconut water indicated that it would probably make a good, acceptable infant food.

Economic considerations, such as copra contracts, constant supply of plant and equipment, have not made the process acceptable to territorial administrations for the present. The plant could be supplied for quite small scale operation but the cash product - the oil - would require drums or tankers for transport to the metropolitan countries and the cost of these, together with high freight rates for both the empty and full drums, also help to make the process an uneconomic one at present.

The coconut protein, however, is an extremely interesting material and is worthy of more intensive investigation as a possible vegetable "milk" and infant food.

An article on the subject has been published in the South Pacific Commission's Quarterly Bulletin (Vol. 3 : 1. 1953).



VII CONCLUSIONS

From the analytical results obtained it would seem that an adequate and balanced diet could be derived from the local foodstuffs available in the Pacific. However, it must be realised that the foods examined were drawn from many parts of the area and therefore they would not all be available in any one territory. In particular is this true of the districts occupied by those people existing on an extremely limited diet such as the sago eaters of New Guinea. The analytical figures obtained for the various sago preparations and the observed intakes of other foods reported by Oomen and Malcolm (20) would indicate that these people are grossly lacking in such essential food constituents as protein. However, there are many other factors that would have to be investigated before any firm conclusions could be made. For example, we do not know the nitrogen balance figure for these people, what their basal metabolic rate may be, how efficiently they utilize all the nutrients that they consume, and in particular their nitrogen intake.

The proteins of taro and coconut could be classed as being good proteins, those of sweet potato as being fair proteins and those of yam as being poor. The protein composition of sweet potato should be further investigated, and it would be interesting to see whether there is any correlation between colour and nitrogen content. On the figures presented, it would seem that there might be a correlation. At the same time however, the amino acid make up of the proteins of yellow and white sweet potato seems to be the same. Much work yet remains to be done on the various taros. No figures are available for *Xanthosoma* sp. or *Cyrtosperma* sp. and a study of the various taro species and their amino acid composition would be very useful.

A study which in the author's opinion should be undertaken, and which was begun in the work reported above, is in reference to the composition of the coconut at all stages of maturity from the "drinking nut" stage to the sprouted nut stage. Time did not allow of the study of the amino acid composition of coconut water at various growth stages. There is a definite change in the sugars and the total nitrogen of the water during maturation and it would be interesting to see if there was any change in the free amino acid content and in the relation of the various amino acids with each other. At present it can be said that the coconut is an extremely valuable food, which should be more thoroughly exploited for this purpose than is now the case. The present tendency appears to be that the food role of the coconut is being largely superseded by its "cash crop" role, and it is being replaced as a food by cassava and similar foods. This tendency is to be deplored and efforts should be made to find other sources of money for the indigenous peoples, so that the incentive to use the coconut as an economic crop would not be as great as it is now.

Much more work will have to be done on the pandanus. The results reported above are at considerable variance with those of Miller (16), especially regarding the carotene content. No previous reference can be found describing the nature of the pigments present in the fruit. It appears to

have been generally accepted that the colour was due to carotenoid pigments and that pandanus was a good source of carotene. It would appear from the results reported that this is not the case, and in fact the pandanus from the Gilbert Islands are a poor source of carotene. Unfortunately the pandanus growing in New Caledonia are of inedible genera and species, and do not appear to colour to the same extent as those present in other parts of the Pacific. Work on this subject could not be undertaken because of the difficulty of obtaining suitable material.

The need for a more intensive investigation into the nitrogen fraction of the foods is indicated. The bibliography on the chemical composition of South Pacific foods shows clearly that no work has yet been done on this most important subject. It is regretted that the establishment of the laboratory and the work necessary before amino acid analyses could be carried out took so long, and that the closing of the laboratory occurred just as this work was beginning to produce results in this field.

The need for a greater knowledge of the amino acid composition of foods is now widely realised, and the laboratory in Noumea was the only one in the South Pacific region doing this work. It is to be hoped that another laboratory will take up this task and continue the work that has now been commenced.

Several of the lesser known foods examined would make valuable additions to the diets of many groups in the South Pacific region if they were introduced. The nuts of *Pandanus julianetti* are a good source of protein, fat and niacin. *Sesbania grandiflora* leaves are an extremely rich source of carotene and ascorbic acid and a fair protein source. Pawpaw leaves are a good protein source and have been reported in the literature as being a rich source of carotene. The pigeon pea is a good protein source. It is unfortunate that more samples of pulses were not sent in for analysis, as these appear generally to be good all-round foods.

The dried fish and smoked breadfruit, both from the British Solomon Islands, are good examples of local practices in food preservation, which could easily be adapted to other parts of the Pacific and which could make available valuable supplementary foods or items of trade.

There is still much scope for the development of a good, cheap, protein concentrate, such as the coconut protein concentrate reported, which could be used as a weaning food and as a general food supplement.

In publishing the results set out above, it is hoped that they will be of value to the various people interested in nutrition in the South Pacific and in the health and well-being of the peoples of this area.

BIBLIOGRAPHIES OF PACIFIC ISLAND FOODSTUFFS

During the course of the investigations into the nutritional value of Pacific Islands foodstuffs, an extensive literature survey was made. The results of this literature survey have been published by the South Pacific Commission.

A Bibliography of the Nutritional Aspects of the Coconut was published as South Pacific Commission Technical Paper No.58 in April 1954, and a revised version as Technical Paper No.95 in September 1956.

An attempt was made to make this bibliography as complete as possible and to cover all aspects of the nutritional value of the coconut. No papers were included, however, which dealt mainly with copra production, oil manufacture or the nutrition and diseases of the coconut palm itself. In the original edition no papers dealing with "toddy", the sap of the coconut palm, were included, but on the advice of Dr. R. Child, the former Director of the Ceylon Coconut Research Station, the nutritional value of toddy was also covered in the revised version.

An Annotated Bibliography of the Composition of Pacific Foods was published as South Pacific Commission Technical Paper No.100 in 1957. This bibliography covers many of the foods used in the South Pacific Region. The selection of the foods covered is based on the suggestions of M. J. Barrau, the Commission's officer investigating subsistence agriculture. Several widely used foods, such as cassava and beans, were not included, however, as extensive bibliographies are already available on these foods, and it was felt that the repetition would not be justified. It is interesting to note at this stage that many of the foods suggested by M. Barrau have not been investigated chemically and therefore no literature is available dealing with them.

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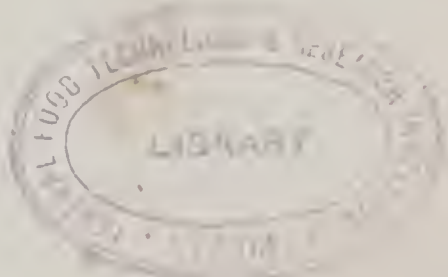
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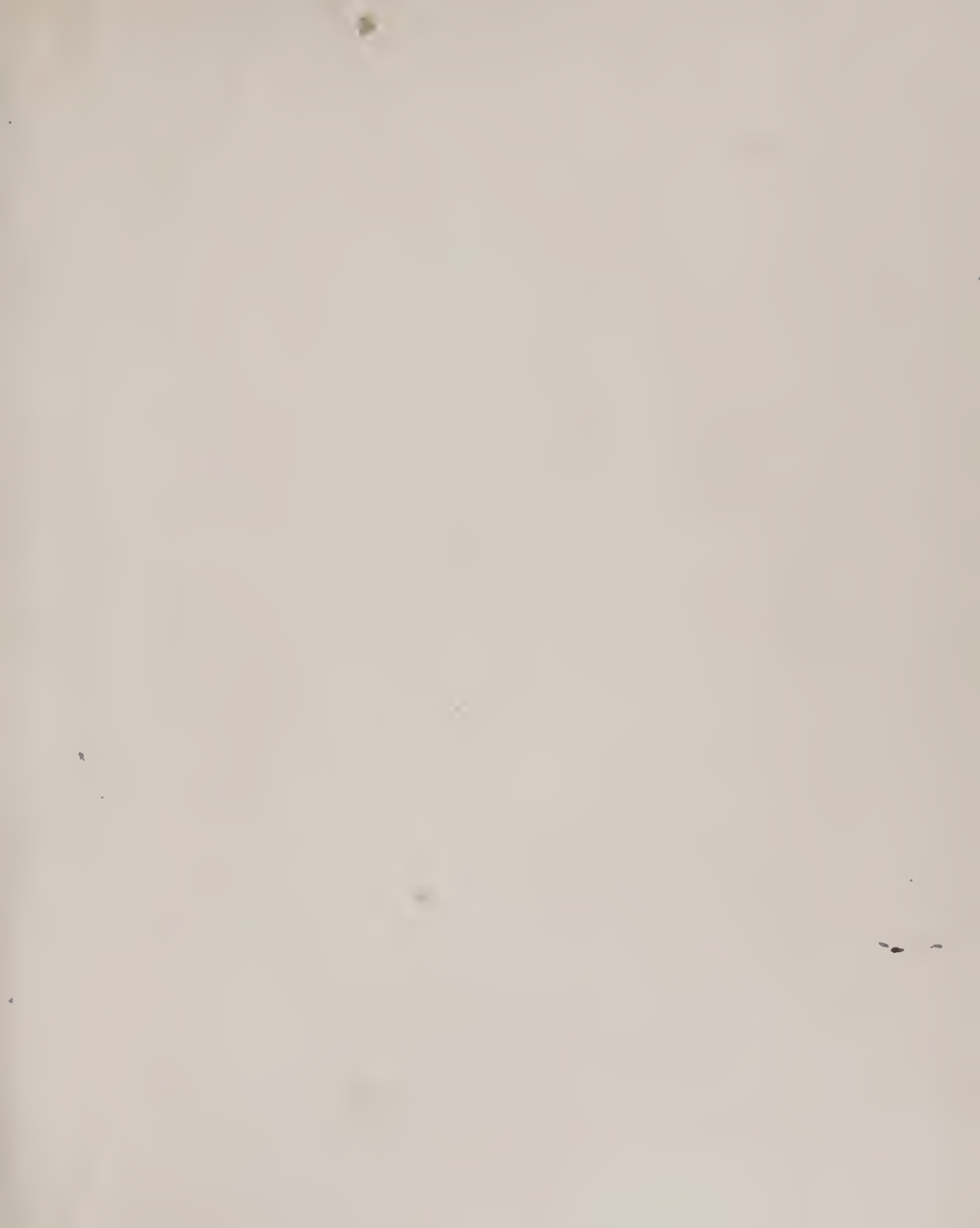
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Appendix 5

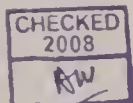
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